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ARCHIVES OF PATHOLOGY

VOLUME 40 JULY 1945 NUMBER 1

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MIXED TUMORS OF THE SALIVARY GLANDS

C. ALEXANDER HELLWIG, M.D.

WICHITA, KAN.

HISTORICAL REVIEW

In 1859 Billroth [1] described the tumors of the salivary glands from the standpoint of surgical pathology so completely that during the eighty-six years that have followed hardly any new details have been added to the anatomic and clinical picture of these tumors. Billroth characterized the tumors, which occurred most frequently in the parotid glands, as painless, slowly growing, often multiple, encapsulated, and soft or hard depending on their relative amounts of mucoid or cartilaginous stroma. While these tumors persistently recurred after surgical removal, metastases were extremely rare. Billroth gave an admirable account of their complex histologic structure as observed in unstained tissue sections. He described not only the reticulate arrangement of the spindle or star-shaped cells and the myxochondroid stroma but also the adenomatous and papillary structures that occurred, sometimes with cyst formation. He called attention to the similarity between some of these tumors and cylindroma. He had first applied the term "cylindroma" to rare tumors of the orbital cavity. Squamous cells, even horn pearls, were present in some of his specimens, and he also observed atypical round cell carcinoma as the final stage in a parotid tumor which in a period of twenty-five years had recurred ten times after as many operations. The only new feature which has since been added to the morphologic picture of these tumors is the occasional occurrence of bone. This was first observed by Virchow [2] in a submaxillary tumor, and the observation was later confirmed by Volkmann,[3] Orth,[4] Hinsberg,[5] Wilms [6] and Borst.[7]

The term "mixed tumor," which now is in general use, was introduced fifty years ago by Minssen [8] in his review entitled "The Mixed Tumors of the Parotid."

Much of the literature on mixed tumors of the salivary glands which has since appeared is made up of repetitious descriptions of histologic details which confirm Billroth's classic description. There has been much disagreement for the last eighty-five years, however, about how to explain the complex histologic structure of these tumors. The problem of the nature and the origin played the principal role in the literature on mixed tumors between 1885 and 1905, and that problem is today as unsolved as it was at the time of Billroth.

Without reviewing the literature in detail I may say that there have been three theories advanced to explain the unique structure of tumors of the salivary glands: (1) that they are mesenchymal derivatives, (2) that they are purely epithelial adenoma and (3) that they are of bidermal origin, true mixed tumors of epithelial and mesenchymal origin.

Mesenchymal Theory.—Billroth regarded the tumors of the salivary glands as myxoma, and Virchow [2] in his treatise on neoplastic diseases called them enchondroma. He contended that they originated from the fibrous stroma and the capsule of the salivary glands. Virchow felt that the epithelium in these tumors was derived from connective tissue elements. All of the early German pathologists regarded the tumors of the salivary glands as derived from connective tissue and supposed any epithelium-like structures to be vestiges of the glands in which they occurred. French pathologists, on the other hand, were inclined to the opinion that they were primarily epithelial and originated in the gland tissue itself.

From the Department of Pathology, St. Francis Hospital, and the Sedgwick County Tumor Clinic.

1. Billroth, T.: Virchows Arch. f. path. Anat. **17**: 357, 1859.

2. Virchow, R.: Die krankhaften Geschwülste, Berlin, A. Hirschwald, 1863, vol. 1, p. 502.

3. Volkmann, R.: Deutsche Ztschr. f. Chir. **41**: 107, 1895.

4. Orth, J.: Lehrbuch der speziellen pathologischen Anatomie, Berlin, A. Hirschwald, 1882, vol. 1, p. 629.

5. Hinsberg, V.: Deutsche Ztschr. f. Chir. **51**:281, 1899.

6. Wilms, M.: Die Mischgeschwülste, Leipzig, A. Georgi, 1902, p. 190.

7. Borst, M.: Die Lehre von den Geschwülsten, Wiesbaden, J. F. Bergmann, 1902, vol. 2, p. 882.

8. Minssen, H.: Ueber gemischte Geschwülste der Parotis, Inaug. Dissert., Göttingen, G. Hofer, 1874.

In 1895 Volkmann expressed the view that he had demonstrated the endothelial origin of the mixed tumors of salivary glands. His teaching spread to all countries and today is still accepted by some pathologists. The adherents of the endothelial theory explained the occurrence of cartilage, mucoid tissue and even squamous cells by a metaplastic versatility of the endothelial parent tissue.

Epithelial Theory.—In 1899 Hinsberg,[5] after a painstaking histologic study of 9 mixed tumors, criticized the endothelial theory. He assumed that the mixed tumors started from embryonic rests of the parotid anlage, which develops as a budlike invagination of the buccal epithelium. Ribbert,[9] Küttner [10] and E. Kaufmann [11] adopted Hinsberg's theory, while Krompecher [12] regarded mixed tumors as varieties of basal cell carcinoma. The epithelial theory is today the leading theory of the histogenesis of the mixed tumors. Of the writers adhering to this view some (Zymbal [13]; Leroux [14]; Dockerty [15]) have accepted an origin from adult glandular epithelium, others (Masson and Peyron [16]; Menétrier and Peyron [17]) have derived the mixed tumors from the embryonal tissue of the salivary glands, while a third group has favored the view that the tumors arise from epithelium detached from the buccal cavity in early embryonal life. This epithelial matrix is believed to have the potency to form squamous as well as glandular epithelium (Wilms [6]). The writers who have adhered to the epithelial origin of mixed tumors have admitted the difficulty of accounting for the most prominent feature of these tumors, namely, the occurrence of mucoid and chondroid stroma. Clementz [18] expressed the opinion that the cartilage in mixed tumors might be formed directly from the epithelial cells. His view was accepted by Ehrich,[19] who stated that the epithelial cells of salivary glands may undergo mucoid degeneration and thus give rise to a substance whose consequent condensation results in cartilage. Masson and Peyron,[20] as well as Ewing,[21] were convinced that the cartilage is a secretory product of the epithelial cells of the salivary gland tumors. Norrenbrock [22] also derived the mixed tumors from the embryonal epithelium of the mouth and explained the myxochondroid stroma by an organizing action of the tumor cells on the surrounding mesenchymal tissue, in accordance with Speman's theory of organization. This view has been accepted in a recent paper by Hemplemann and Womack.[23]

Bidermal Theory.—Cohnheim [24] in his lectures on general pathology wrote that the enchondroma of the parotid region consists of unappropriated particles derived from the cartilaginous portions of the branchial arches. Twenty years later his view was revived by Cuneo and Veau.[25] Basing their view on the embryology of the neck and a study of 6 cervical tumors, these authors stated that mixed tumors arose as the result of "enclavement," or sequestration, of some of the embryonal material of the branchial arches. McFarland [26] accepted this theory and explained the puzzling histologic peculiarities of mixed tumors by the number and the character of the originally detached materials. He compared the embryonal material of these "enclavomas" to plants in a badly arranged garden in which some grow rapidly and suppress the more delicate flowers. Çhevassu [27] derived most salivary gland tumors from misplaced elements of the branchial arches; however, he pointed out other possible sources, namely, adult and embryonal glandular epithelium and even true teratoma.

A summary of the three main theories of the origin of the tumors of the salivary glands shows that in each one difficulties arise in explaining all the different constituents of these tumors. If the epithelial origin is accepted, the occurrence

9. Ribbert, H.: Geschwülstlehre, Bonn, F. Cohen, 1914, vol. 2, p. 601.

10. Küttner, H.: Beitr. z. klin. Chir. **16**:181, 1896.

11. Kaufmann, E.: Lehrbuch der speziellen pathologischen Anatomie, Berlin, W. de Gruyter & Co., 1908, p. 382.

12. Krompecher, E.: Beitr. z. path. Anat. u. z. allg. Path. **44**:51, 1908.

13. Zymbal, W. E.: Beitr. z. path. Anat. u. z. allg. Path. **91**:113, 1935.

14. Leroux, R.; Leroux-Robert, J., and Dillange, P.: Bull. Assoc. franç. p. l'étude du cancer **22**:490, 1933.

15. Dockerty, M. B., and Mayo, C. W.: Surg., Gynec. & Obst. **74**:1033, 1942.

16. Masson, P., and Peyron, A.: Bull. Assoc. franç. p. l'étude du cancer **7**:219, 1914.

17. Menétrier, P., and Peyron, A.: Bull. Assoc. franç. p. l'étude du cancer **11**:503, 1922.

18. Clementz, H.: Ueber das Schleimgewebe in Parotisgeschwülsten, Inaug. Dissert., Bonn, C. Georgi, 1882.

19. Ehrich, E.: Beitr. z. klin. Chir. **51**:368, 1906.

20. Masson, P., and Peyron, A.: Bull. Assoc. franç. p. l'étude du cancer **6**:219, 1913.

21. Ewing, J.: Neoplastic Diseases, ed. 4, Philadelphia, W. B. Saunders Company, 1940, p. 789.

22. Norrenbrock, K. W.: Zur Histogenese der Parotis-Mischgeschwülste, Leipzig, G. Thieme, 1931, p. 63.

23. Hemplemann, L. H., and Womack, N. A.: Ann. Surg. **116**:34, 1942.

24. Cohnheim, J.: Pathologie, Berlin, A. Hirschwald, 1882, vol. 1, p. 736.

25. Cuneo, G., and Veau, V.: Cong. internat. de méd. (sect. de chir. gén.) **10**:278, 1900.

26. McFarland, J.: Am. J. M. Sc. **132**:804, 1926; **203**:502, 1942; Surg., Gynec. & Obst. **76**:23, 1943.

27. Chevassu, M.: Rev. de chir., Paris **41**:145, 1910.

TABLE 1.—*Data on Eighty-Two Cases of Neoplastic Involvement of the Salivary Glands*

Case	Age	Sex	Location	Histologic Features
			1. MIXED TUMOR TYPE	
			A. Abundant Stroma	
1	25	F	Parotid	Fibromyxoid stroma; squamous cells, solid strands of cells
2	..	F	Parotid	Myxochondroid stroma; solid strands of cells; one cyst
3	..	F	Parotid	Fibromyxoid stroma; solid and cystic areas
4	56	F	Submaxillary	Fibromyxoid stroma; solid strands of cells, few squamous cells
5	..	F	Parotid	Myxochondroid stroma; solid strands, few glands
6	53	F	Parotid	Myxochondroid stroma; narrow strands of cells
7	..	F	Parotid	Myxochondroid stroma; solid strands, few glands
8	30	F	Parotid	Myxochondroid stroma; solid strands, few glands
7	..	F	Parotid	Myxochondroid stroma; solid strands, few glands
8	30	F	Parotid	Myxochondroid stroma; solid strands, few glands
9	25	F	Submaxillary	Myxochondroid stroma; solid strands, few glands
10	69	F	Parotid	Chondroid stroma; few narrow strands of cells
11	63	F	Parotid	Myxochondroid stroma; narrow strands of cells
12	..	M	Parotid	Myxochondroid stroma; narrow strands, few glands
13	51	F	Parotid	Myxochondroid stroma; narrow strands of cells
14	..	M	Parotid	Myxochondroid stroma; few glands. narrow strands
15	30	M	Parotid	Myxochondroid stroma; narrow strands of cells
16	54	F	Parotid	Myxochondroid stroma; narrow strands of cells
17	60	F	Parotid	Myxochondroid stroma; solid strands of cells.
18	..	F	Parotid	Myxochondroid stroma; solid strands, few lumens
19	56	F	Parotid	Myxochondroid stroma; solid strands
20	41	F	Parotid	Myxochondroid stroma; solid masses of cells
21	77	F	Parotid	Myxochondroid stroma; solid strands, few squamous cells
22	38	F	Parotid	Myxochondroid stroma; solid strands of cells
23	67	M	Parotid	Myxochondroid stroma; solid strands, few lumens
24	28	M	Submaxillary	Myxochondroid stroma; solid strands. few glands
25	..	F	Parotid	Myxochondroid stroma; few glands and cysts
26	22	F	Parotid	Myxochondroid stroma; narrow strands of cells
27	..	M	Parotid	Myxochondroid stroma; solid strands, few lumens
28	18	F	Parotid	Myxochondroid stroma; solid strands, few squamous cells
29	30	M	Parotid	Myxochondroid stroma; narrow strands, few lumens
30	44	F	Parotid	Myxochondroid stroma; solid strands, few lumens
31	..	F	Parotid	Chondroid stroma; narrow strands of cells
32	36	F	Parotid	Myxochondroid stroma; solid strands of cells
33	64	F	Parotid	Myxochondroid stroma; solid strands
			B. Marked Epithelial Proliferation	
34	53	M	Submaxillary	Mucoid stroma; solid groups of cells, squamous cells
35	24	M	Parotid	Myxochondroid stroma; glands, squamous cells with horn pearls
36	42	F	Parotid	Myxochondroid stroma; solid masses of cells
37	..	M	Submaxillary	Myxochondroid stroma; solid and glandular
38	30	F	Submaxillary	Myxochondroid stroma; solid groups of cells, squamous cells with horn pearls
39	50	F	Submaxillary	Mucoid stroma; solid groups of cells
40	20	M	Palate	Scanty mucoid stroma; solid groups of cells
41	35	F	Palate	Myxochondroid stroma; solid and glandular
42	..	M	Parotid	Hyaline stroma; solid groups of cells, few lumens
43	..	F	Parotid	Scanty mucoid stroma; solid and glandular
			C. Atypical Proliferation	
44	..	F	Parotid	Myxochondroid stroma; squamous cells
45	31	F	Parotid	Mucoid stroma; atypical cells
46	64	M	Parotid	Myxochondroid stroma; solid groups of cells, squamous cells, few glands
47	55	M	Parotid	Myxochondroid stroma; alveolar and squamous cell proliferation
48	63	M	Submaxillary	Resemblance to squamous cell carcinoma
49	70	M	Parotid	Resemblance to spindle cell sarcoma
50	29	M	Parotid	Myxochondroid stroma with atypical spindle cells
51	45	M	Parotid	Solid masses of spindle cells, few glands
			2. ADENOMATOUS TYPE	
			A. Papillary Cystadenoma Lymphomatosum	
52	42	M	Parotid	
53	40	M	Parotid	
			B. Adenoma	
54	53	F	Parotid	Glandular proliferation with cyst formation
55	..	F	Palate	Cystadenoma
56	62	F	Submaxillary	Small glandular structure; narrow canals
			C. Papillary Adenoma	
57	..	M	Parotid	
58	14	M	Parotid	
59	..	F	Parotid	
60	14	F	Parotid	
61	62	M	Submaxillary	
62	50	M	Parotid	
63	..	M	Parotid	
			D. Cylindroma	
64	..	M	Parotid	
65	31	F	Parotid	
66	..	M	Submaxillary	
			E. Basiloma	
67	..	M	Parotid	
			3. COMBINATION OF ADENOMATOUS AND MIXED TYPE	
68	37	F	Parotid	Myxochondroid stroma; solid strands and cylindroma
69	52	M	Parotid	Myxochondroid stroma; solid strands and cylindroma
70	..	F	Parotid	Chondroid stroma; adenomatous and squamous cell proliferation
71	68	M	Parotid	Myxochondroid stroma; basiloma
72	73	M	Parotid	Myxochondroid stroma; basiloma
73	70	F	Parotid	Myxochondroid stroma; adenomatous proliferation
74	..	F	Parotid	Myxochondroid stroma; papillary cystadenoma and cylindroma
75	..	M	Parotid	Myxochondroid stroma; cylindroma and adenoma
			4. CARCINOMA	
76	67	M	Parotid	Lymphoepithelioma
77	72	M	Submaxillary	Lymphoepithelioma
78	61	M	Parotid	Adenocarcinoma
79	80	M	Parotid	Squamous cell carcinoma
80	48	F	Parotid	Lymphoepithelioma
81	45	M	Parotid	Transitional cell carcinoma
82	62	M	Parotid	Squamous cell carcinoma

of cartilage is hard to account for. The mesodermal theory gives no ready explanation of the epithelial structures, especially the glandular and the squamous epithelium. The bidermal theory leaves unanswered the question of why, if the tumors take origin from multipotent embryonal cells of the ectoderm and the mesoderm, the differentiation regularly leads to the characteristic picture of mixed tumors.

MATERIAL

This paper is based on a study of 82 primary tumors of the salivary glands. From each of 4 patients 2 specimens were obtained on different occasions, and from 1 patient, 3 specimens. The clinical data were abstracted and compared with the gross and microscopic observations. The microscopic study was made on paraffin sections representing two or three different blocks of each tumor. The routine stain was hematoxylin and eosin. In selected cases other stains were employed: Van Gieson, Mallory's connective tissue stain, orcein and thionine.

In table 1 the material is arranged according to age and sex of patient and location and histologic character of tumor.

GENERAL OBSERVATIONS

Incidence.—Mixed tumors of the salivary glands are not common. McFarland found that in the course of twenty-five years only 310 mixed tumors were removed in the six largest hospitals of Philadelphia, a yearly average of 2 for each hospital. The patients were operated on by seventy-three surgeons, an average of 3.2 patients for one surgeon. Thirty-seven of the seventy-three surgeons each had only 1 patient with a tumor of this type during twenty-five years. Schreiner and Mattick[28] in analyzing the records of 6,695 patients of the State Institute for the Study of Malignant Diseases, Buffalo, found that 66 had tumors of the salivary glands, a tumor incidence of 1 per cent. Ahlbom,[29] of Radiumhemmet, which attracts tumor patients from all over Sweden, reported a series of 254 tumors of the salivary glands covering a twenty year period. Leroux and Leroux-Robert[30] collected 102 mixed tumors of the salivary glands and the palate from the material sent to the Faculty of Medicine in Paris. Harvey, Dawson and Innes[31] had 253 salivary gland tumors in the material of the Research Laboratory of the Royal College of Physicians in Edinburgh. At the Memorial hospital for the Treatment of Cancer and Allied Diseases, New York, Pack and LeFevre[32] saw 202 mixed tumors among 3,827 tumors of the oral and the nasal cavity, a percentage of 5 per cent.

The 82 primary tumors of the salivary glands on which my study is based were observed during a period of twenty years, among 4,352 cancers. The largest number of mixed tumors examined during a single year was 12, in 1940. The incidence of 2 per cent agrees well with the figures given by Billroth, who found 40 tumors of the salivary glands among 2,058 cancers, or 1.94 per cent.

Age.—Tumor of the salivary glands may occur at any age. No congenital tumor of this type has been reported. Duthoit,[33] however, saw a tumor of the parotid gland in a child 6 weeks of age, and Wood's[34] youngest patient was 7 months old. Among Ahlbom's patients with such tumors the age limits were 9 to 84 years. Heineke,[35] who collected 428 tumors of the salivary glands from the literature, gave an average age of 29 years for patients with noncancerous, and 49 years for those with cancerous, mixed tumors of the salivary glands. Stein and Geschickter[36] stated that most of their patients with mixed tumors were between 20 and 40 years of age and that relatively more of their patients with cancerous growths were over 45 years. In Ahlbom's series the average age of the 130 patients with benign and semimalignant tumors was 43 years, while for the 124 patients with malignant tumors the corresponding age was 52 years. McFarland noted the peak of incidence late in the third decade. In my series the average age was 44.7 years for patients with mixed tumors, 38.4 years for those with adenomatous tumors, and 62.1 years for those with cancers. The youngest patient was 14 years old and the oldest 80 years.

Sex.—It is generally stated in the literature that mixed tumors show no predilection regarding sex. Heinecke, Pack and LeFevre found a definite predominance of men over women among patients with cancerous tumors of the salivary glands of the nonmixed type. Ahlbom reported a rather even sex distribution for his whole series, namely, 106 males and 148 females.

28. Schreiner, B. F., and Mattick, W. L.: Am. J. Roentgenol. **21**:541, 1929.

29. Ahlbom, H. E.: Acta radiol., 1935, supp. 23, p. 1.

30. Leroux, R., and Leroux-Robert, J.: Bull. Assoc. franç. p. l'étude du cancer **23**:304, 1934.

31. Harvey, W. F.; Dawson, E. K., and Innes, J. R. M.: Debatable Tumors in Human and Animal Pathology, Edinburgh, Oliver & Boyd, 1940, p. 17.

32. Pack, G. T., and LeFevre, R. G.: Am. J. Cancer **14**:167, 1930.

33. Duthoit, cited by Ahlbom.[29]

34. Wood, F. C.: Ann. Surg. **39**:57 and 207, 1904.

35. Heineke, H.: Ergebn. d. Chir. u. Orthop. **6**:254, 1913.

36. Stein, I., and Geschickter, C. F.: Arch. Surg. **28**:492, 1934.

In the group with noncancerous tumors there was a definite predominance of women, while in the group with cancers more men than women were affected. He expressed the belief that the prevalence of women in the group with noncancerous tumors was not a real one but was due to the fact that more women than men come for treatment of their lesions because they are more concerned about the disfigurement caused by the growth.

My own series substantiates the general observation that more women are operated on for mixed tumors of the salivary glands (37 to 22 males) but that cancerous tumors of these glands occur more frequently in men (6) than in women (1). Among the patients with adenomatous types there were 9 males and 6 females.

Location of Tumor.—Since 1892, when Böhme [37] reviewed reports of 411 salivary gland tumors in the literature, the preference of these tumors for the parotid glands has been well known. Böhme gave the following relative frequency of location: parotid glands 90.5 per cent, submaxillary glands 8.5 per cent and sublingual glands 1.2 per cent. His statistics were confirmed by those of Wood and of Schreiner and Mattick. In Ahlbom's series the tumors of the parotid glands were fourteen times more frequent than those of the submaxillary glands. Only 2 of his 254 mixed tumors were located in the sublingual glands; 22 were found in the palate and 5 in the pharyngeal wall. The figures given by McFarland are: parotid glands 93.6 per cent, submaxillary glands 6.1 per cent and sublingual glands 0.5 per cent. They represent an unselected distribution of these tumors. Harvey, Dawson and Innes with a series of 275 tumors found 230 located in the parotid glands, 21 in the submaxillary glands and 2 in the sublingual glands. Six tumors originated in the palate.

In my material I encountered 67 tumors of the parotid glands (82 per cent), 12 of the submaxillary glands and 3 palatal tumors. No mixed tumors were found in the sublingual glands.

The striking preference of the mixed tumors for the parotid glands has been almost completely overlooked by the writers who have attempted to clarify the origin of mixed tumors. The current theories of their histogenesis certainly do not explain this peculiar anatomic predilection.

37. Böhme, H.: Inaug. Dissert., Berlin, 1901; cited by Wolff, J.: Die Lehre von der Krebskrankheit, Jena, G. Fischer, 1911, vol. 2, p. 554.

HISTOLOGY

According to McFarland, the primary tumors of the salivary glands can be divided conveniently into two main groups: first, the interstitial mixed type, in which mucoid, hyaline and chondroid tissue preponderates, and, second, the glandular type, with predominance of epithelial elements. Dockerty [15] divided the primary tumors of the submaxillary glands into (1) adenocarcinoma of the mixed tumor type and (2) adenocarcinoma of the cylindroma type, (3) a combination of these two types and (4) miscellaneous. My grouping of the 82 tumors is similar; however, I found it necessary to add several subgroups. A comparison of my material with that reported by McFarland, Dockerty and Mayo, Stein and Geschickter, and Ahlbom shows good agreement on the relative frequency of the different tumor types (table 2). More than 70 per cent of the specimens present the mixed tumor type, 20 per cent are of glandular structure and less than 10 per cent are true cancers. I believe that the first two types, the mixed and the adenomatous, are closely related and that there is no sharp line of distinction between them. Not infrequently a combination of both types occurs in the same specimen, and a tumor removed by operation may belong to the mixed type and its recurrence to the adenomatous type.

TABLE 2.—*Incidence of Different Types of Salivary Gland Tumors*

Type	McFarland [26]	Dockerty [15]	Ahlbom [29]	Sheldon *	Hellwig
Mixed tumor type	149	51	135	25	51
Adenomatous type	56	15	55	19	16
Combination	..	7	..	..	8
Carcinoma	13	8	24	7	7
Total number of cases	218	81	214	51	83

* Sheldon, W. H.: Arch. Path. 35:1, 1943.

The clinical behavior of mixed tumors differs from that of adenomatous tumors considerably. McFarland found little difference in the percentage of recurrence after operation between the various types of mixed tumors, regardless of the number or the type of cells (34 to 38 per cent). For the adenomatous group, including cylindroma and papillary adenoma, the rate of recurrence was almost twice as high (60 per cent). Dockerty found a recurrence rate of 36 per cent in his first group and 60 per cent in the second. In McFarland's experience, while mixed tumors, in spite of recurrence, never were the direct cause of death, 45 per cent of the papillary adenomatous tumors caused death, and all the true cancers proved fatal.

In the main, mixed tumors consist of undifferentiated polyhedral or spindle-shaped cells arranged in solid masses, anastomosing strands or islands of closely packed cells. The nucleus of the tumor cell is hyperchromatic, and a nucleolus is seldom visible. The scanty cytoplasm is acidophilic. Mitoses are as a rule absent. The tumor cells may form acini with one or several layers of epithelium. Some of the cell complexes may resemble basal cells or may form cysts with mucoid content. In another direction the tumor cells may differentiate into epidermoid cells, producing typical squamous cells with intercellular spines and even horn pearls.

The more or less abundant myxoid, hyaline and chondroid stroma gives these tumors their special character. From close histologic study one sees that the cartilaginous substance is not an inclusion of a displaced anlage of cartilage, as in true teratoma, since it lacks a perichondrium. On the contrary, it is evident that the myxochondroid stroma develops by transformation or by secretory function of the epithelial tumor cells. The mucoid substance is found first in vacuoles of the cytoplasm of some of the epithelial cells and then appears in the close surrounding of the cells. The supporting tissue thus becomes mucoid by imbibition of the epithelial product. It stains metachromatically with thionine blue; sometimes it gives a hyaline staining reaction. Apparently hyaline and mucoid changes are only phases of the same process which terminates in the production of chondroid substance. In the process epithelial cells tend to become isolated in a retraction space, which gives them all the appearance of cartilage cells within their capsules. The vagueness of the boundary between epithelial tumor cells and the cells of mesenchymal appearance is the outstanding characteristic of mixed tumors. Figure 8 shows how the epithelial cells become narrower, separate from one another and gradually take on the form of the scattered cells of the myxochondroid tissue. The epithelial cells and the cells of mesenchymal appearance in mixed tumors are apparently of identical origin.

NATURE AND ORIGIN OF MIXED TUMORS

My histologic observations substantiate the contention of Clementz,[18] Erich,[19] Masson and Peyron [20] and Ewing [21] that in mixed tumors of the salivary glands the myxochondroid stroma is derived from the epithelium. The problem of the histogenesis of these tumors therefore hinges about the origin of cells which can appear as of epithelial and again as of mesenchymal nature.

Ewing [21] tried to explain this dual behavior of the cells in mixed tumors by stating that they originate in glands which form an exceptional class because they produce mucin and secrete ptyalin. This view is not consistent with the location of mixed tumors. The parotid glands, in which mixed tumors are most common, do not produce mucin, while the submaxillary and sublingual glands which secrete mucin, are rarely the site of mixed tumors. Also on histologic grounds Ewing's view seems untenable. Ewing himself pointed out that the small mixed tumor does not exhibit any relations to the ducts or the acini of the gland in which it is located. Dockerty,[15] who expressed the view that mixed tumors are adenocarcinoma originating from differentiated epithelium of salivary glands, was unable to find in any case an actual point of origin from glandular epithelium. Also Harvey, Dawson and Innes [31] admitted that the mixed tumors appear constantly walled off from the normal gland by dense fibrous tissue. According to McFarland,[26] there is little resemblance between the epithelial arrangements seen in the tumors and in the salivary glands; the mixed tumors are in but not of the gland.

In 1918 Forman and Warren [38] expressed a hypothesis which gives a much better explanation of the nature and the origin of mixed tumors. These authors derived mixed tumors from mesenchyme, which originates from ectoderm. One of the authors demonstrated that in the urodeles cartilage can be derived from epithelium. This is a confirmation of the work of Marshall,[39] who recognized that some of the epithelial cells of the neural crest in the head region went into the formation of mesenchyme. In 1893 Platt [40] stated that in Necturus the branchial cartilages are formed from cells whose origin can be traced back to ectoderm. This observation has been substantiated for some of the fishes and amphibians by Kupffer, Lundborg and Dohrn.[41] Forman and Warren expressed the belief that demonstration of such tissue in mammals would offer a ready explanation for the peculiar structure of mixed tumors. Inclusion or misplacement of such an ectodermal mesenchyme could give rise to tumors the cells of which either fail to differentiate and simulate the epithelial cells from which they arose or they differentiate along the lines they normally do and form myxoid, chondroid, connective tissue and sometimes bone.

38. Forman, J., and Warren, J. H.: Ann. Surg. **47**: 67, 1918.

39. Marshall, A. M.: Quart. J. Micr. Soc. **18**:10, 1878.

40. Platt, J. B.: Anat. Anz. **8**:507, 1893.

41. von Kupffer, C.; von Lundborg, H., and Dohrn, A., cited by Forman and Warren.[38]

THE PRIMITIVE NOTOCHORD

While studying the intervertebral disk in human embryos histologically, I was impressed with the similarity in structure between the fetal disk and the mixed tumors of salivary glands. The intervertebral disk develops from the notochord. It seems to be the only ectodermal tissue in mammals which differentiates from epithelial into myxochondroid tissue. The question therefore arises: Is the notochord perhaps the embryonal ectodermal tissue Forman and Warren suspected of giving rise to mixed tumors?

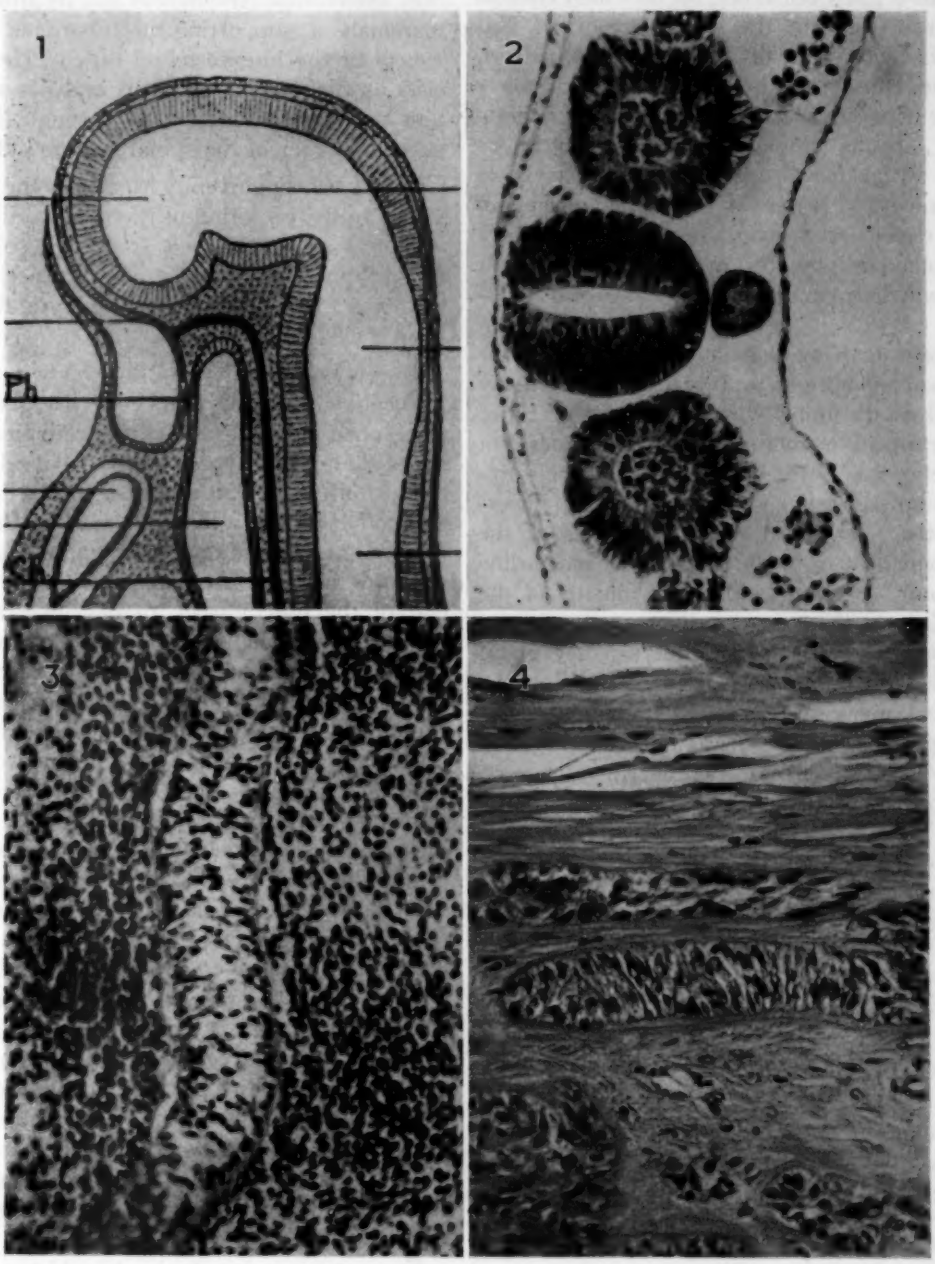

Fig. 1.—Diagram of the head of a 6 mm long rabbit embryo. *Ch* indicates the notochord attached to the pharyngeal membrane (*Ph*) (Hertwig [48]).

Fig. 2.—Cross section of a 48 hour old chicken embryo. Note the epithelial notochord adjacent to the embryonal foregut.

Fig. 3.—Notochord (nucleus pulposus) of a 13 mm. long human embryo. It is an epithelial column with a hyaline sheath.

Fig. 4.—Mixed tumor of a parotid gland, imitating the epithelial stage of the notochord.

Origin and Fate of the Notochord.—The origin of the notochord is ascribed by Piraud [42] to the ectoderm. The opinion of Hertwig,[43] Maximow [44] and Ewing [21] that the structure is derived from entoderm is explained by the fact that during an early stage of development the chorda dorsalis is temporarily enclosed in that tissue and forms a thick cell layer, the so-called chordal plate, in it, beneath the neural groove. This ridge of cells gradually becomes pinched off from the entoderm and first forms a hollow tube, the chordal canal, and finally a solid column of cells. In its cranial portion the notochord extends as far forward as the buccopharyngeal membrane (fig. 1). As the oral pocket of Seessel develops, the notochord remains for a time attached to its posterior wall, and, as Linck [45] has shown, it even enters the pharyngeal membrane.

In the fourth week of embryonal life, the pharyngeal membrane ruptures, and the embryonal oral cavity unites with the pharynx. Since the pharyngeal membrane sometimes does not completely disappear, but persists in the form of membranous remnants at the original insertion of the pharyngeal membrane, cells of the chorda which were in the pharyngeal membrane may become scattered with the remnants of the latter (Coenen [46]).

The parotid gland is the first salivary gland to develop. One week after rupture of the pharyngeal membrane a keel-like flange forms by invagination of the buccal epithelium in the region where ectoderm and entoderm meet, i. e., at the former site of the pharyngeal membrane (Arey [47]). Should there be any cells of the notochord scattered during rupture of the pharyngeal membrane and should they be lying in the path of the developing parotid gland, it is conceivable that they could be carried along by the budding gland to its final location and could give rise to tumors in or near it. The same process may explain the much less frequent occurrence of mixed tumors in the region of the submaxillary gland. Moral [48] demonstrated that during one stage of development the submaxillary glands are in close proximity to the parotid glands. The occurrence of mixed tumors in the palate, the third common site, can also be explained by an origin from chordal cells, since the palate develops where the cranial portion of the notochord lies adjacent to the epithelial lining of the foregut.

Histology of the Development of the Notochord.—While the notochord constitutes the only skeleton of Amphioxus and its allies, among mammals a supporting notochord is transient except at the intervertebral disks. Here it persists as the nucleus pulposus and has an important mechanical function all through life as the shock absorber of the spinal column (Beadle [49]).

I had the opportunity to study the different stages in the evolution of the notochord on intervertebral disks obtained from gynecologic and autopsy material. My youngest specimen was from a 13 mm. long human embryo; the oldest, from an 80 year old man.

In an embryo of 2.4 mm. body length, described by His, the notochord has the appearance of a hollow tube with a distinct lumen (Keibel and Mall [50]). The epithelial cells around the primitive lumen are in a single layer and of cuboidal form. In a later stage the chorda consists of a solid cylindric column composed of large cells with clear protoplasm. At the end of the fourth week of development a thin hyaline sheath encircles the chorda. In midfetal life the nucleus pulposus forms a syncytium of slender undifferentiated oxyphilic cells, surrounded by a mucoid fluid. In early extrauterine life a gradual swelling of the network with fluid is observed. The nuclei are forced apart, remaining joined only by fine strands. Many of the cells become vacuolated and are lost by dissolution during this process. After termination of growth, the intercellular stroma becomes myxochondroid in character and overshadows the cells. The syncytium of slender chordal cells is no longer present, and the nucleus pulposus of the adult consists of a homogeneous mass with only few scattered cells of cartilage-like appearance. In old age, fibrillary degeneration of the matrix and fine fissuring of the intervertebral disk are observed. By loss of fluid a compact but easily fissuring cartilage-like dry substance results, in which isolated large cells are occasionally found.

From the study of the life history of the nucleus pulposus the following stages can be distinguished: (1) a hollow tube, then a solid

42. Piraud, G.: La notochorde, Paris, E. Le François, 1933.
43. Hertwig, O.: Die Elemente der Entwicklungslehre, Jena, G. Fischer, 1910.
44. Maximow, A. A.: A Textbook of Histology, Philadelphia, W. B. Saunders Company, 1930, p. 21.
45. Linck, A.: Anat. Hefte **42**:605, 1911.
46. Coenen, H.: Beitr. z. klin. Chir. **133**:1, 1925.
47. Arey, L. B.: Developmental Anatomy, Philadelphia, W. B. Saunders Company, 1937, p. 179.
48. Moral, H.: Anat. Hefte **47**:277 and 383, 1913.
49. Beadle, O. A.: The Intervertebral Disc, London, His Majesty's Stationery Office, 1931.
50. Keibel, F., and Mall, F. P.: Manual of Human Embryology, Philadelphia, J. B. Lippincott Company, 1910.

column of epithelial cells; (2) a syncytium of undifferentiated cells; (3) cytoplasmic vacuolation and secretion of mucinous fluid into the intercellular spaces; (4) transformation of cells and stroma into a myxochondroid tissue.

The similarity between the histologic appearances that mark the development of the notochord and the peculiar structure of mixed tumors is obvious. The salient features of mixed tumors are syncytial undifferentiated cells, epithelial and

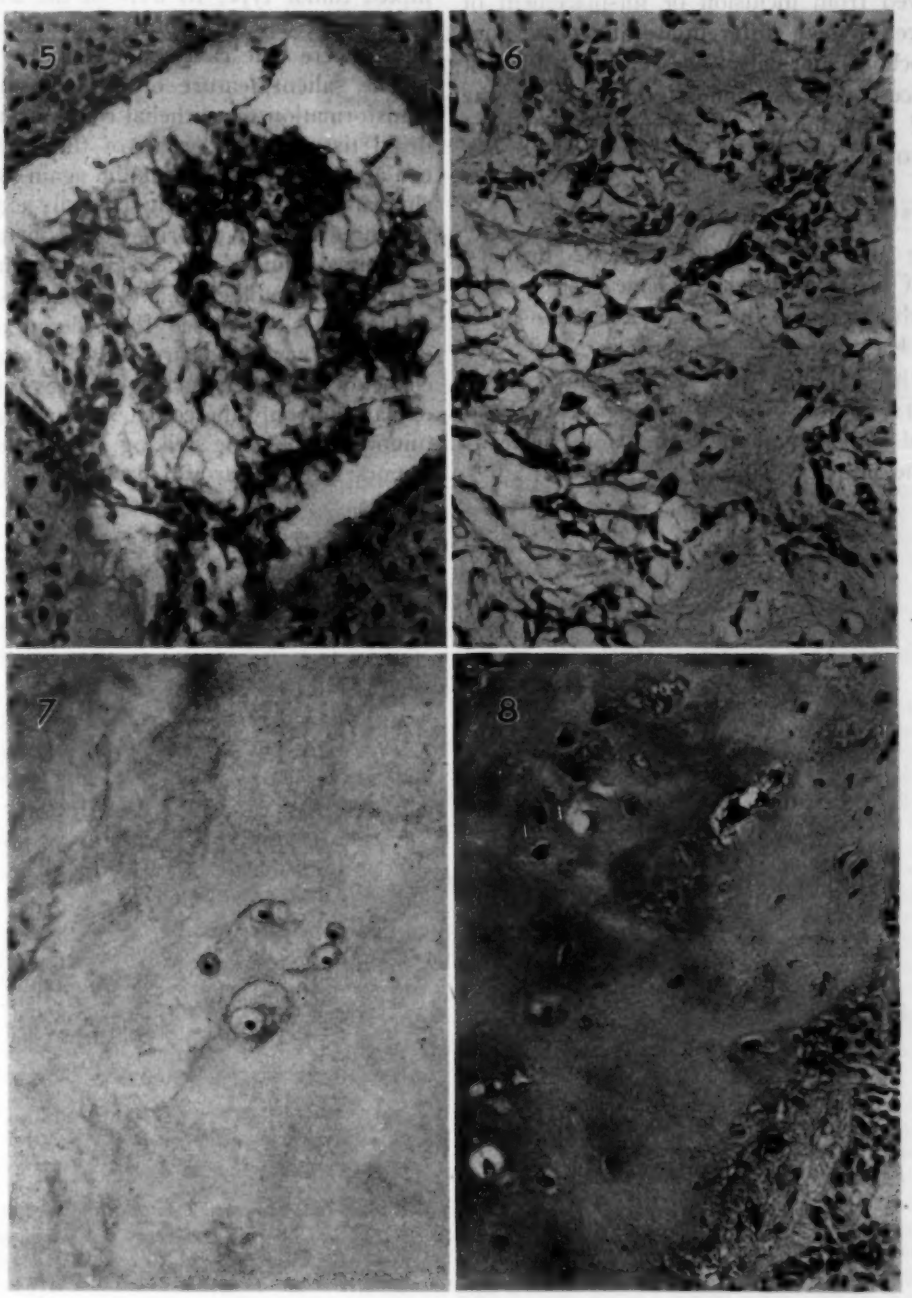

Fig. 5.—Notochord of a 13 cm. long human fetus. It is a syncytium of undifferentiated cells. Vacuolation and extracellular production of mucoid substance may be observed.

Fig. 6.—Mixed tumor imitating the midfetal stage of the notochord. It shows a network of undifferentiated cells. Note the vacuolation and the extracellular production of mucoid substance.

Fig. 7.—Notochord (nucleus pulposus) of a 48 year old female. It shows a chondroid matrix with cartilage-like cells.

Fig. 8.—Mixed tumor of a parotid gland. Note the transition of epithelium into cartilage. The cartilage-like stroma and cells imitate the adult stage of the nucleus pulposus (notochord).

adenomatous structures, mucin secretion and transformation into myxochondroid tissue. These characteristics of mixed tumors, especially the transformation of epithelium into cartilage, can be explained without difficulty if these tumors are derived from inclusion or misplacement of chordal cells which differentiate along the lines the notochord normally does. The different appearances of mixed tumors correspond to the stages of evolution of the primitive notochord (figs. 2 to 8).

SUMMARY AND CONCLUSIONS

During the last twenty years 82 primary tumors of the salivary glands have been studied in the laboratory of the St. Francis Hospital, Wichita, Kan., among 4,352 cancers. The average of the patients with mixed tumors was 38.4 years. More women were operated on for mixed tumors and more men for true cancers of the salivary glands.

Sixty-eight (82 per cent) tumors were located in the parotid glands, 12 in the submaxillary glands, 3 in the palate and none were encountered in the sublingual glands.

Histologically, 51 of the 82 tumors were of mixed tumor type, 16 were of the adenomatous type, 8 presented combinations of these 2 types and 7 were true cancers.

The salient feature of mixed tumors is the transformation of epithelial cells into myxochondroid tissue. The origin of tumor cells which can appear as of epithelial and again as of mesenchymal nature is not satisfactorily explained by the three current theories of the histogenesis of the mixed tumors of the salivary glands.

From embryologic, histologic and topographic studies it is concluded that the mixed tumors of the salivary glands are derived from misplaced elements of the notochord. This view would explain not only the complex structure of mixed tumors but also the striking predilection of these tumors for the parotid glands.

HEMATIC AND ANATOMIC CHANGES IN DOGS AFTER REPEATED INTRAVENOUS INJECTIONS OF LARGE AMOUNTS OF INULIN SOLUTION

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Since the introduction of inulin clearance tests for the determination of glomerular filtration in 1934 and 1935 (Richards, Westfall and Bott,[1] Shannon and Smith[2]) the parenteral introduction of inulin dissolved in isotonic solution of sodium chloride has become a diagnostic procedure commonly used in clinical and experimental investigations (Hueper[3a]). It is apparent from the numerous reports dealing with the medicinal use of such solutions that properly prepared, pyrogen-free, sterile solutions of inulin do not seem to elicit any untoward reactions in man and animals. Shannon and Smith[2] injected intravenously into man amounts up to 160 Gm. of inulin without observing any subjective or objective disturbances. It is a well established fact, moreover, that parenterally injected inulin is practically completely eliminated in the urine within twenty-four hours. However, apart from these observations there are no systematic studies on the potential hazards of the repeated intravenous introduction of this agent. Since it is also interesting to compare the hematic and anatomic effects elicited by inulin after repeated intravenous administration with those induced by larger macromolecular colloids (polyvinyl alcohol, methyl cellulose, pectin, acacia, sodium cellulose glycollate, gelatin, ovalbumin) (Hueper[3b]), the following experimental studies were made.

EXPERIMENTAL PROCEDURE AND RESULTS

Inulin, having a molecular weight of 3,000 to 5,000, is a polymer of levulose (Drew and Haworth[4]; Haworth, Hirst and Percival[5]; Berner[6]). It does not give a blue reaction with iodine as starch does and gives a negative reaction in Benedict's and Fehling's tests. As the organism does not possess a mechanism for the metabolic degradation of the inulin molecule, the substance does not serve as a source of energy but is excreted intact. Inulin filters through collodion membranes which withhold proteins (Heupke and Blankenburg[7]; Richards, Westfall and Bott[1]). The inulin used in the present experiments was isolated from dahlia tubers. A pyrogen-free, sterile 20 per cent solution of inulin in 0.65 per cent sodium chloride solution was used.

Four dogs were given intravenous injections of this solution. The weights of the dogs varied between 9 and 11 Kg., and the ages ranged from 7 months to 2.5 years. For the study of the acute hematic reactions 1 dog was given 10 cc. of the 20 per cent inulin solution, 1 dog 30 cc. and 2 dogs each 150 cc. The blood was examined (hemoglobin, erythrocytes, leukocytes, sedimentation rate, volume of packed blood cells) directly before the injection and thirty minutes, forty-five minutes, three hours, four hours, five hours and twenty-four hours after it.

During the second phase of the experiments in which the effects of repeated intravenous injections on the blood were determined, 2 dogs forty-eight hours after the first injection received daily injections of 50 cc. of the 20 per cent inulin solution. After five such injections the daily dose was increased to 75 cc. (five times per week), and after another set of five injections it was raised to 100 cc. Two weeks later the dose was elevated to 150 cc. and kept at this level until the end of the experiment. The 2 dogs which had received 150 cc. of inulin solution at their first injection remained on this dose during the entire course of the experiment. The duration of the experiments varied for the different dogs from fifty to sixty-five days (fifty, fifty-one, fifty-seven, sixty-five). The total amount of inulin solution administered ranged from 4,155 to 6,750 cc. for the different dogs, containing 831, 850, 923 and 1,350 Gm. of inulin, respectively. The previously mentioned studies of the blood and determinations of the viscosity of the plasma were made on these dogs at weekly intervals.

On several occasions the body temperature of each dog was taken at frequent intervals after the injection (forty-five minutes, three hours, five hours, twenty-four hours) to ascertain the absence of pyrogenic reactions. No such reactions were observed. Three of the dogs declined about 1 Kg. in weight during the course of the experiments. The fourth dog showed only minor fluctuations in weight. During the last two weeks of the experiments the intake of water of 1 of the 2 dogs which had received 150 cc. of inulin solution from the start was restricted to the amount of water introduced with the solution and with the food, altogether not more

From Warner Institute for Therapeutic Research.

1. Richards, A. N.; Westfall, B. B., and Bott, P. A.: Proc. Soc. Exper. Biol. & Med. **32**:73, 1934.

2. Shannon, J. A., and Smith, H. W.: J. Clin. Investigation **14**:393, 1935.

3. Hueper, W. C.: (*a*) To be published; (*b*) Arch. Path. **33**:267, 1942; (*c*) Am. J. Path. **18**:895, 1942.

4. Drew, H. D. K., and Haworth, W. N.: J. Chem. Soc., 1928, pt. 2, p. 2690.

5. Haworth, W. N.; Hirst, E. L., and Percival, E. G. V.: J. Chem. Soc., 1932, pt. 2, p. 2384.

6. Berner, E.: Ber. d. deutsch. chem. Gesellsch. **63**: 1356, 1930.

7. Heupke, W., and Blankenburg, K.: Deutsches Arch. f. klin. Med. **176**:182, 1933.

than 500 cc. This procedure was instituted to increase the concentration of inulin in the urine and possibly also in the blood and to favor thereby the precipitation and retention of inulin in the renal tubules. The voided urine of these animals contained large masses of precipitated inulin, which gave the urine a creamy appearance. The urine of these dogs, varying in amount between 134 and 297 cc. daily, showed a high specific gravity (up to 1.060), an alkaline reaction, traces of albumin and no reduction with Benedict's reagent.

One dog died, showing at autopsy bilateral purulent pleurisy, congestion and hemorrhagic indurations of the lung, and a contracted, thickened and highly congested bladder wall. The lumen of the bladder contained a considerable amount of a yellowish white cheesy or starchy material, which gave a distinct inulin reaction. Each of the other 3 dogs was killed by an intravenous injection of a 4 per cent solution of formaldehyde. The internal organs of these dogs were grossly normal.

The acute hematic reactions following the intravenous injection of 10 to 150 cc. of a 20 per cent inulin solution did not conform to any consistent pattern. In the 2 dogs given 20 cc. and 30 cc., respectively, there occurred a moderate drop in the number of erythrocytes during the first three hours, amounting to 700,000 cells in each animal. Following a brief, incomplete recovery of the erythrocytic values at the four hour examination, there occurred a second drop which persisted at the twenty-four hour examination, amounting to 600,000 and 1,200,000, respectively. Hemoglobin and hematocrit values exhibited parallel fluctuations. There were no significant alterations in the number of leukocytes and in the sedimentation rate. The 2 dogs which received 150 cc. as an initial dose did not display any appreciable changes of these factors with the exception of a distinct reduction in the number of erythrocytes and in the amount of hemoglobin at the twenty-four hour examination, amounting to a decrease of 800,000 to 1,000,000 cells and of 2 to 4 Gm. of hemoglobin.

During the chronic phase of the experiment, in all 4 dogs the erythrocytes and the hemoglobin exhibited a slow but progressive drop reaching at times a loss of more than 2,000,000 cells. The dog which during the last two weeks of the experiment was placed on a water-restricted diet revealed during this stage a partial recovery of its erythrocytic values apparently through

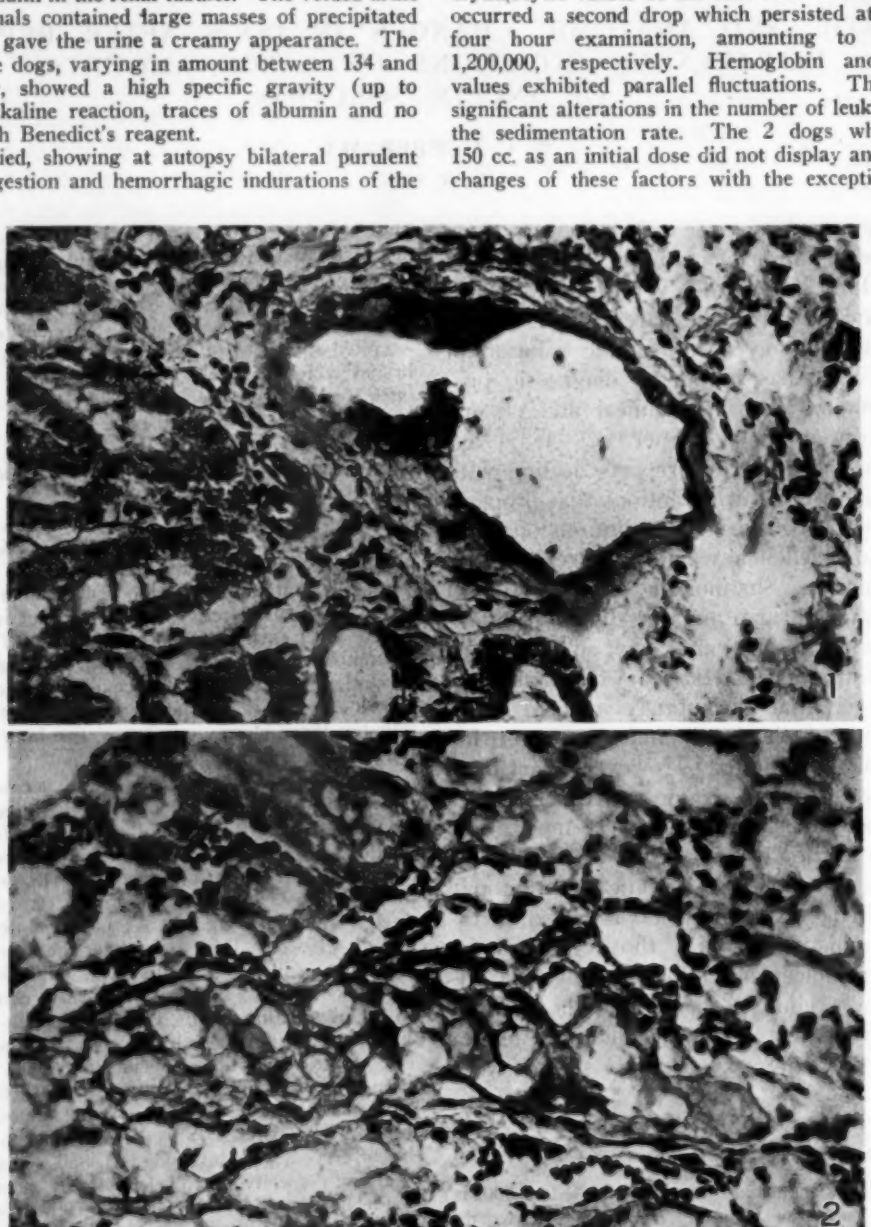

Fig. 1.—Tubule with distended lumen lined by a syncytial and giant cell nuclear mass.
Fig. 2.—Tubular lumens filled with foam cells.

hemoconcentration. There were no significant or appreciable changes in the number of leukocytes, the sedimentation rate and the viscosity of the plasma.

The inulin content of the plasma was determined in these dogs on various occasions by the method of Hubbard and Loomis.[8] Determinations were made directly before the injection of 150 cc. of inulin solution and thirty minutes, forty-five minutes, three, four, five and twenty-four hours afterward. The peak value seen at the thirty minute test ranged between 200 and 600 mg. per hundred cubic centimeters of blood; at the five hour test, the value dropped to almost original levels. There was no retention of inulin in the blood during the period of restricted intake of water according to the twenty-four hour tests run repeatedly during this time.

The histologic examination of the internal organs of the 4 dogs revealed renal changes attributable to the administration of inulin only in 1. In this animal the cortical tubules showed in places moderately distended lumens lined by a syncytial and giant cell structure surrounding an almost unstained reflecting material. In the environmental interstitial tissue there were mononuclear cells and large cells with clear cytoplasm (figs. 1 and 2). All other organs of this animal and all the organs of the remaining 3 dogs were essentially normal with the exception of extensive leukocytic infiltrations and intra-alveolar edema and hemorrhages in the lungs of the dog that died from a pulmonary condition. The bladder of this animal exhibited a small cystic area of hyperplasia of the epithelial lining and mucosal defects filled by an edematous and hyaline connective tissue, engorged capillaries, scattered hemorrhages and small infiltrations with mononuclear cells. There were no atheromatous changes in the blood vessels and no foam cell structures in the internal organs, such as the liver, the kidney, the adrenal gland, the lung and the spleen.

COMMENT

The acute hematic changes seen after the intravenous injection of a solution of inulin are evidently the result of fluctuations in the water content of the blood brought about by the osmotic disturbances set up there by the injected inulin. This explains the reduction in the number of erythrocytes during the first twenty-four hours after the injection. It is noteworthy that there were no colloidoclastic phenomena (leukopenia, increased sedimentation rate) in any of the animals. The absence of these reactions was also prominent during the chronic phase of the experiments, indicating that the relatively small colloidal particles of inulin do not elicit in the blood responses the same as those associated with the intravenous introduction of other macromolecular colloids (gelatin, pectin, methyl cellulose, acacia, polyvinyl alcohol). The reduction in the number of erythrocytes observed during the latter part of the chronic phase may be a result of hydremia or it may be an anemic response. A definite decision in this matter cannot be made because of the absence of blood volume determinations. That it was the result of hydremia, however, is made likely by the fact that there was observed a partial recovery of the erythrocytic values in the dog whose intake of water was restricted.

The deposits observed in the renal tubules of 1 dog, which were probably composed of precipitated inulin and which elicited a foreign body reaction, suggest that a disproportion between the amount of urine and the concentration of inulin in the glomerular filtrate may cause the inulin to fall out of solution. That such a reaction is rather unusual is indicated by the fact that in the dog whose intake of water was restricted these lesions did not develop.

The absence of any signs of retention of inulin in the vascular walls and the viscera under the formation of foam cells is evidence that such phenomena are related to the molecular size and shape of the colloidal matter introduced into the organism. Previous investigations with another colloidal carbohydrate possessing a chain molecule, methyl cellulose (Hueper [3e]), have shown that molecules of an average weight of about 30,000 are retained in the body and give rise to visceral storage reactions. Thus the borderline of the permeability of membranes for colloidal and macromolecular material of this type must lie between molecular weights of 30,000 and 6,000.

SUMMARY

The intravenous injection of large amounts of inulin dissolved in 0.65 per cent saline solution elicits hydremic phenomena during the first twenty-four hours after injection. The hydremic conditions may become persistent when daily injections are given over a period of two months.

In only 1 of 4 dogs did the renal tubules show inulin casts which elicited a foreign body reaction.

There was no evidence of colloidoclastic responses in the blood of the treated animals or of storage phenomena in the vascular walls and internal organs of any of the dogs.

The daily repeated intravenous injection of a sterile and pyrogen-free solution of inulin, administered over a period of two months, did not elicit any serious untoward reactions in the blood or the internal organs.

8. Hubbard, R. S., and Loomis, T. A.: J. Biol. Chem. **145**:641, 1942.

THE BRAIN IN LEUKEMIA

A CLINICOPATHOLOGIC STUDY OF TWENTY CASES WITH A REVIEW OF THE LITERATURE

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Although involvement of the central nervous system with attending neurologic signs and symptoms is not usually thought of as a frequent complication of leukemia, Schwab and Weiss [1] reported that the incidence of neurologic signs and symptoms in a series of 334 cases of leukemia collected from several Boston hospitals was 20 per cent. In that investigation it was not possible to study adequately and correlate the neurologic symptoms and signs observed with the type and the extent of the pathologic changes in the central nervous system, since in only 3.3 per cent of the cases was there postmortem examination of the central nervous system and in none of these was the brain carefully sectioned. Several anatomic studies (Diamond [2]; Hamburger [3]; Hellich [4]; Veszprémi [5]) of the brain in leukemia have indicated that some pathologic change occurs in this organ in a high percentage of instances, but none of these studies included a sufficient number of cases to allow adequate clinical correlation with the observed pathologic changes.

The present report contains a clinicopathologic study of 20 cases of leukemia in which a pathologic examination of the brain was made at the Washington University School of Medicine. The study appeared worth while in view of the relatively few cases that have had clinicopathologic study. In order to broaden the study and better evaluate the incidence of pathologic changes occurring in the brain in leukemia and correlate them with the neurologic signs and symptoms, the previously reported cases having sufficiently adequate pathologic study to be of value have been collected and reviewed.

From the Department of Pathology of the Washington University School of Medicine, St. Louis.

1. Schwab, R. S., and Weiss, S.: Am. J. M. Sc. **189**:766, 1935.

2. Diamond, I. B.: Arch. Neurol. & Psychiat. **32**: 118, 1934.

3. Hamburger, W.: Frankfurt. Ztschr. f. Path. **46**: 257, 1933.

4. Hellich, I.: Deutsche Ztschr. f. Nervenh. **128**: 278, 1932.

5. Veszprémi, D.: Virchows Arch. f. path. Anat **184**:220, 1906.

MATERIAL AND METHODS

The 20 brains utilized for this study were from patients with leukemia who had complete clinical and anatomic study. There was 1 patient with leukosarcoma, but patients with other related diseases, such as Hodgkin's disease, chloroma and lymphosarcoma without leukemic manifestations, were excluded. The brains, collected from 1939 to 1944 and fixed and stored in 4 per cent solution of formaldehyde were from patients observed in the Barnes Hospital and the St. Louis Children's Hospital and were taken at random, there being no attempt to select or discard a case because the patient did or did not have neurologic signs and symptoms. For the determining of the gross pathologic changes the brains were sliced with a standard electrical slicing machine. By this method it was possible to section the brain serially, the individual slices being not more than 3 to 5 mm. in thickness; thus was offered an unusual opportunity to observe accurately the destribution and the extent of the pathologic changes.

After a careful examination of the sliced brain to determine the extent and the distribution of grossly visible lesions, blocks of tissue were taken for histologic study from all grossly visible lesions and from representative parts of the right half of the brain as suggested by Spielmeyer.[6] The sections routinely taken were from the first frontal gyrus, the central gyri, the first temporal gyrus, the insula, the corpus striatum, the cornu ammonis, the calcarine fissure, the optic thalamus, the hypothalamus, the mesencephalon in the region of the nuclei of the oculomotor nerves, the pons in the region of the nucleus of the abducens nerve, the medulla at the level of the inferior olive, the vermis of the cerebellum and the cerebellar hemisphere.

The blocks of tissue selected for histologic study were stained with hematoxylin and eosin. For the demonstration of Nissl substance in the nerve cells and the obtaining of the best cytologic detail the routine sections in 2 cases (63 and 65 in the accompanying table) were stained with thionine. The tissue blocks in case 65 were fixed in the 4 per cent solution of formaldehyde used in the other cases, but the blocks in case 63 were fixed in 80 per cent alcohol.

6. Spielmeyer, W.: Technik der mikroskopischen Untersuchung des Nervensystems, ed. 3, Berlin, Julius Springer, 1924, p. 29.

REPORT OF CASES

A brief clinical history, the results of the pathologic study of the brain and the final anatomic diagnosis for each of the 20 cases forming the basis of this study are presented here. All of the brains were given the detailed gross and microscopic study previously outlined, but only the significant pathologic changes in each are recorded.

CASE 1.—Chronic subleukemic myeloid leukemia in a 60 year old white woman with only slight pathologic change in the brain, who showed changes in the deep reflexes shortly before death.

The onset of the final illness occurred one year before death, at which time hematologic studies gave a white blood cell count characteristic of a subleukemic phase of myeloid leukemia. Both the red blood cell count and the hemoglobin content were low, and a biopsy of the marrow showed great hyperplasia of the cells of the myeloid series. The spleen and the liver were slightly enlarged. On the final entry into the hospital, three weeks before death, the red blood cell count was 4,200,000, and the hemoglobin content was 62 per cent. The white blood cell count had risen to 56,400, and the platelet count was 46,000. The differential count revealed 3 monocytes, 8 lymphocytes, 24 segmented polymorphonuclear leukocytes, 1 stab form, 5 metamyelocytes, 17 type C myelocytes, 32 type B myelocytes, 9 type A myelocytes and 1 myeloblast. Depression of the achilles and patellar reflexes, noted two weeks before death, was the only neurologic sign. The patient died in shock following a blood transfusion one year after the onset of the leukemic disease.

Postmortem Examination of the Brain.—The weight of the brain was 1,250 Gm. Petechiae were noted in the dura mater and in the cerebral cortex and the basal ganglions in the serial sections taken through the brain.

Histologic examination revealed a moderate degree of leukostasis in the blood vessels of the brain in all the sections taken. In a large number of the sections the perivascular spaces contained scattered red blood cells and leukemic cells in moderate numbers. Single or multiple small hemorrhages were present in the parenchyma in the sections taken from the central gyri, the caudate nucleus, the temporal gyrus and the substantia nigra. The hemorrhages were most numerous in the substantia nigra.

Pathologic Diagnosis.—Myeloid leukemia; leukemic hyperplasia of the marrow; leukemic infiltrations of the liver, the spleen, the kidneys, the epicardium, the adrenal glands, the lymph nodes and the pancreas; petechiae in the dura mater, the brain, the skin, the conjunctiva, the pleura, the pericardium and the mucosa of the stomach and the bladder; bilateral hydrothorax.

CASE 2.—Acute myeloid leukemia in a 14 year old white girl with significant pathologic change in the brain but with only slight disturbance of vision.

Four months prior to admission to the hospital the patient had noted increasing weakness, loss of weight and petechiae in the skin, and for one month before entry there had been frequent elevations of temperature and severe nosebleeds. After admission, the hemorrhagic manifestations of the disease became increasingly severe, and focal hemorrhages were noted in the ocular fundi. Terminally there were blurring of vision, dizziness and mental confusion. The white blood cell count varied between 50,000 and 125,000; 50 to 75 per cent of the cells were granulocytes, as determined by the peroxidase reaction. The differential count revealed 32 early blast forms, 16 juvenile forms and 52 lymphocytes. The red blood cell count was slightly above 1,000,000, and the number of platelets was decreased. Treatment was ineffectual, and the patient died two months after her admission to the hospital.

Postmortem Examination of the Brain.—The brain weighed 1,200 Gm. There were foci of hemorrhage in the subarachnoid space at points scattered over both cerebral hemispheres, the largest measuring 5 mm. in diameter. Serial sections through the brain revealed a focus of hemorrhage 4 cm. in diameter in the left temporal lobe. Smaller foci of hemorrhage were encountered in the left internal capsule and in the cerebellum.

Histologic examination revealed leukostasis in the blood vessels, accumulations of leukemic cells in the perivascular spaces and irregularly outlined foci of leukemic cells in the brain tissue in all sections. A section from the white matter of the left temporal lobe adjacent to the large focus of hemorrhage noted in the gross examination disclosed that the focus was surrounded by an accumulation of leukemic cells 7 mm. in diameter. In the cerebral cortex two smaller foci of hemorrhage were encountered that were connected by a broad zone of leukemic cells in the tissue.

Pathologic Diagnosis.—Myeloid leukemia; leukemic hyperplasia of the marrow; leukemic infiltrations of the brain, the lungs, the spleen, the lymph nodes, the liver, the kidneys and the heart; hemorrhage in the left temporal lobe of the brain; focal hemorrhages in the subarachnoid space, the cerebral cortex, the internal capsule and the cerebellum, the skin, the lungs, the kidneys and the mucosa of the urinary bladder.

CASE 3.—Chronic myeloid leukemia in a 32 year old white man, with slight pathologic change in the brain and no neurologic signs or symptoms.

For two years prior to the final admission to the hospital, the patient had noted great fatigability and a loss of general body strength. It was known that he had chronic myeloid leukemia for one year prior to his final admission to the hospital, there being a greatly elevated white blood cell count, with many myeloblasts and blast forms seen in the blood smear. The spleen had been enlarged for one year but had been fairly well controlled by irradiation until two months before death, when this organ became tremendously enlarged. Because of abdominal pain, severe headaches and a continual elevation of temperature, he was admitted to the hospital. Laboratory studies revealed 3,800,000 red blood cells per cubic millimeter, with the hemoglobin content 63 per cent. The white blood cell count was 68,000, and the differential count disclosed 55 myelocytes, 10 band forms, 31 segmented polymorphonuclear leukocytes and 4 monocytes. The presenting symptoms on entry were not alleviated by treatment in the hospital, which included radiation therapy, and his condition grew steadily worse. The white blood cell count reached an all time high of 214,000 cells shortly before death. At no time during the illness did the patient have any neurologic signs or symptoms.

Postmortem Examination of the Brain.—There was a moderate increase in the weight of the brain, to 1,550 Gm. A slight cerebellar pressure cone with herniation of the cerebellar tonsils into the foramen magnum along the medulla was present. Foci of hemorrhage were seen in the subarachnoid space on the under surface of the frontal lobes, and a 5 mm. focus

of grayish brown discoloration was noted in the gray matter of the occipital portion of the cortex on the right.

Histologic examination revealed leukostasis in the blood vessels of the brain and the meninges. A section taken from the focus of discoloration noted in the occipital portion of the cortex on the right revealed extensive leukostasis in the blood vessels with large numbers of leukemic cells in the perivascular spaces, and in one part of the section there were infiltrations of the leukemic cells in the tissue. Small subarachnoid hemorrhages that extended into the perivascular spaces in the cortical gray matter were noted in the section taken from the frontal lobe, and a small hemorrhage was seen in the pons.

Pathologic Diagnosis.—Myeloid leukemia with leukemic hyperplasia of the marrow, leukemic infiltrations of the brain, the spleen, the liver and the lymph nodes; extensive leukostasis in all tissues examined; infarcts of the spleen; focal hemorrhages in the subarachnoid space, the pons, the gums, and the mucosa of the stomach, the jejunum, the ileum and the renal pelves; fatty degeneration of the myocardium; congestion and edema of the lungs, focal atelectasis of the lower lobes of the lungs; a moderate cerebellar pressure cone.

CASE 4.—Subacute monocytic leukemia in a 38 year old white woman with significant pathologic change in the brain but few neurologic signs.

A year and a half prior to her admission the patient had become increasingly irritable, and for two months before admission she had been acutely ill. Her symptoms had been anorexia, nausea, fever and jaundice. She had largely recovered from this acute illness but had failed to regain her normal strength. Shortly before entering the Barnes Hospital, subcutaneous tender nodules were noted in the skin over the buttocks. On admission, punctate hemorrhages were observed in the ocular fundi and on the lips. There was slight papilledema, and the Babinski and Oppenheim responses were elicited. The cervical and the inguinal lymph nodes were enlarged. The temperature taken rectally was 39.8 C. (103.6 F.). The red blood cell count was 2,170,000, the hemoglobin content was 7.6 Gm. and the platelet count was 34,000. A white blood cell count revealed 80,000 cells, and in the differential count 92 per cent of the cells were young monocytes. Biopsy of the sternal marrow revealed replacement of much of the marrow by monocytic cells. In spite of repeated transfusions, the red blood cell count dropped, and the white blood cell count increased. Subcutaneous hemorrhages developed all over the body, accompanied by profuse bleeding from the nose, the vagina and the rectum. The patient died in coma one week after admission and eighteen months after the onset of her disease.

Postmortem Examination of the Brain.—The weight of the brain was normal, being 1,250 Gm. Gross examination of the brain revealed scattered foci of subarachnoid hemorrhage over the convexities of the cerebral hemispheres and over the cerebellum. In the serial sections through the brain large foci of hemorrhage were seen in the frontal lobe, the basal ganglions, the internal capsule, the substantia nigra, the pons and the occipital lobes. The largest focus of hemorrhage was in the left frontal lobe; it involved the medial part near the central fissure and extended 2 cm. into the centrum ovale. There was much necrosis of tissue in the involved part. The other foci of hemorrhage were less than 5 mm. in diameter. In general, the size of the hemorrhages was smaller in the structures of the hindbrain.

Histologic examination disclosed extensive leukostasis in the blood vessels in all sections studied. Focal lesions of the brain tissue were noted in all the sections. In certain instances the lesion consisted of irregularly outlined accumulations of leukemic cells, usually surrounded by a rim of pale-staining parenchyma (fig. 2). Lesions of this type were seen principally in the corpus callosum and in the mesencephalon. A second type of lesion, usually larger than the aforementioned type, was found in the sections from the frontal lobe and the pons; it consisted of large collections of leukemic cells containing foci of hemorrhage and foci of necrosis of the tissue. Still another type of pathologic change was noted in the sections from the basal ganglions and the frontal lobes and was characterized by focal necrosis of the tissue with diffuse infiltrations of leukemic cells (fig. 5).

Pathologic Diagnosis.—Monocytic leukemia with leukemic hyperplasia of the marrow and the lymph nodes; leukemic infiltrations of the brain, the spleen, the liver, the kidneys and the lungs; focal hemorrhages in the subarachnoid space and in all parts of the brain, the kidneys, the skin, the upper lobe of the right lung and the endometrium of the uterus; hemorrhage with encephalomalacia of the left frontal lobe of the brain; infarcts of the spleen; partially organized thrombus of the superior sagittal sinus; perirenal abscess.

CASE 5.—Subacute lymphoid leukemia in a 5 year old white boy with insignificant pathologic change in the brain and no symptoms of neurologic disease.

Although a clinical diagnosis of aplastic anemia had been made on each of three previous hospital admissions over a period of nine months before the final admission, the diagnosis of lymphoid leukemia was definitely established before death. On the fourth and final admission to the St. Louis Children's Hospital, the white blood cell count had risen to 33,000, and the differential count revealed more than 90 per cent immature lymphocytes, with many blast cells seen. There was at this time general enlargement of the superficial lymph nodes. Many ecchymotic spots were observed in the skin and the legs. The liver and the spleen were palpable. Although the previous course of the disease had been accompanied by intermittent elevations of body temperature, the temperature remained normal for the greater part of this final period in the hospital except for the last week of illness, when it rose to 40 C. (104 F.). The total white blood cell count dropped remarkably before death, but there was no appreciable change in the differential count. The patient died two weeks after his final admission and nine months after the onset of the leukemic disease. Neurologic signs or symptoms were never noted.

Postmortem Examination of the Brain.—The weight of the brain was moderately increased, being 1,400 Gm. Slight opacity and thickening were noted in the leptomeninges over the tips of the frontal lobes and over the inferior surfaces of the occipital lobes. Foci of subarachnoid hemorrhage were scattered over the convexities of the cerebral hemispheres.

Histologic examination revealed marked leukostasis in the blood vessels of all the sections taken. In the section from the thalamus and the internal capsule the perivascular spaces contained moderate numbers of leukemic cells.

Pathologic Diagnosis.—Lymphoid leukemia; leukemic hyperplasia of the lymph nodes and the marrow; leukemic splenomegaly and hepatomegaly; subarachnoid hemorrhage; icterus; thrombosis of the perirectal arteries and veins; infarction of the rectum; congestion and edema of the lungs.

CASE 6.—Acute myeloid leukemia in a 49 year old white woman in whom hemiplegia developed the day before death and pathologic examination revealed an

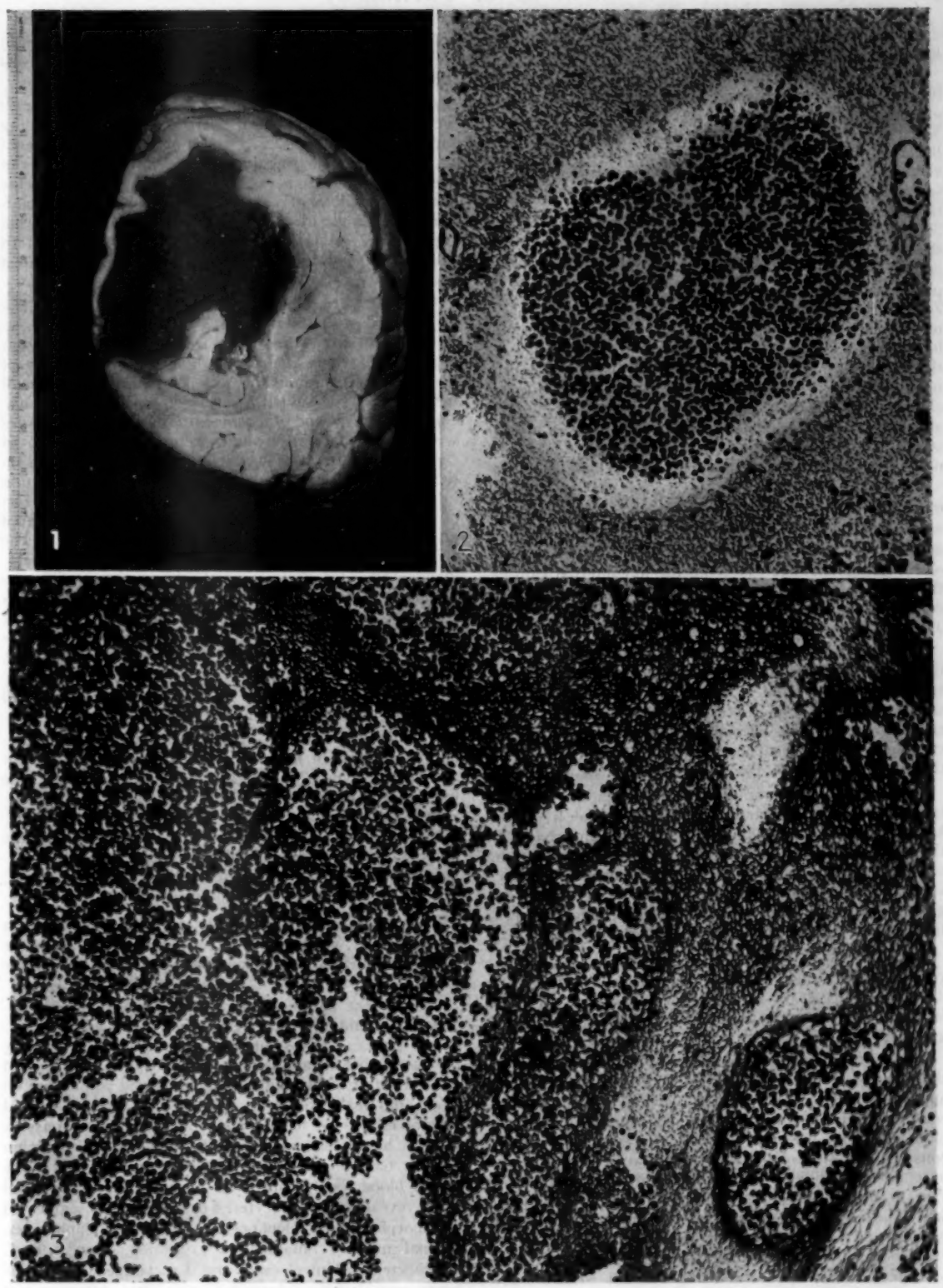

Fig. 1 (case 18 in the table).—Irregularly outlined focus of leukemic cell infiltration and hemorrhage in the right occipital lobe. Note that the lesion shows a remarkable tendency to involve the white matter and to spare the cortical gray matter.

Fig. 2 (case 4 in the table).—Solitary focus of leukemic cells without hemorrhage. Note the pale-staining zone of necrotic white matter surrounding the focus. Hematoxylin and eosin; × 140.

Fig. 3 (case 18 in the table).—Coalescing foci of leukemic cell infiltration in the white matter of the occipital lobe. The white matter surrounding the infiltrating cells is necrotic, and the fibers are broken and disarranged. Hematoxylin and eosin stain; × 140.

extensive hemorrhage in the left internal capsule and basal ganglions.

Before admission the patient for one week had suffered from severe parietal headache, chills and fever and had noticed purpuric spots in her skin and gums. When admitted, she was acutely ill and drowsy, and her temperature was 40.3 C. (104.5 F.). The patellar reflexes were not elicited, and both the achilles reflexes were weak. The red blood cell count was 2,700,000; the hemoblobin content, 8.8 Gm.; the platelet count, 300,000. The white blood cell count was 131,500, and the differential count showed 4 C type myelocytes, 7 metamyelocytes, 4 band forms, 5 segmented polymorphonuclear leukocytes, 2 lymphocytes, 4 monocytes and 74 blast forms. The da, before death the patient failed to respond, and neurologic examination showed the right biceps reflex absent, with Hoffmann's sign present on the right side. Cellulitis of the left arm did not allow testing of the deep reflexes, but Hoffmann's sign was elicited. Patellar and achilles reflexes were not obtainable on either side, and there was no response to plantar stimulus. The patient died, never regaining consciousness. The total duration of the disease was one month.

Postmortem Examination of the Brain.—The weight of the brain was normal, 1,270 Gm. Nothing unusual was noted in the examination of the external surfaces and the leptomeninges. Serial sections revealed a focus of recent hemorrhage involving the left internal capsule, the globus pallidus and other adjacent structures including the anterior nuclei of the thalamus, measuring 7 by 3 cm.

Histologic examination disclosed marked leukostasis in the blood vessels of all sections examined. In the section taken from the frontal lobe the perivascular spaces contained moderate numbers of leukemic cells. The section taken from the focus of hemorrhage in the left internal capsule showed recent hemorrhage with disruption of the brain tissue and beginning necrosis of the tissue.

Pathologic Diagnosis.—Myeloid leukemia; leukemic infiltrations of the marrow, the spleen, the liver, the kidneys, the skin and the lymph nodes; hemorrhages in the lungs; petechiae in the conjunctivas, the skin, the serous membranes and the mucosa of the urinary bladder; infarcts of the kidneys; recent hemorrhage in the left globus pallidus and the internal capsule; fatty degeneration of the myocardium; bilateral hydrothorax.

CASE 7.—Acute subleukemic myeloid leukemia in a 34 year old white man in whom hemiplegia developed the day before his death and whose brain showed a large focus of recent encephalomalacia involving the left basal ganglions.

For approximately six weeks prior to his admission to the Barnes Hospital, the patient noted a hemorrhagic-appearing rash over his chest and arms. A routine blood count revealed leukopenia with neutropenia. He had a chill one week previous to entry that was followed by constant elevation of the body temperature, accompanied by cough with bloody sputum and vomiting. On admission to the hospital, the patient was acutely ill, there was marked pallor of the skin and the mucous membranes, and he was dyspneic on slight exertion. The red blood cell count was 1,100,000, the hemoglobin content 47 per cent and the platelet count 81,000. The white blood cell count was always low and varied from 880 to 4,100 cells. Differential counts revealed 10 segmented polymorphonuclear leukocytes, 18 lymphocytes and 72 myelocytes. In the terminal stage of his illness the patient vomited frequently, his respirations were of the Cheyne-Stokes type, the blood pressure fell and the pulse rate reached 160 per minute. The patient became stuporous the day before his death, and there were noted at that time paralysis of the right half of the face and a flaccid type of paralysis of the right arm and the right leg. He died on the sixth hospital day, seven weeks after the onset of his disease.

Postmortem Examination of the Brain.—The brain weighed 1,340 Gm. There was slight fibrous thickening of the leptomeninges, and there were small, scattered foci of subarachnoid hemorrhage over the convexities of the cerebral hemispheres. Serial sections through the brain revealed an irregular focus of recent encephalomalacia without hemorrhage involving the left internal capsule, the caudate nucleus and the adjacent part of the globus pallidus, measuring 9 cm. in its greatest diameter.

Histologic examination disclosed moderate numbers of red blood cells in the perivascular spaces in the sections taken from the right frontal lobe, the mesencephalon, the pons, the medulla and the softened part of the left basal ganglions. In the last-named section small hemorrhages occasionally were seen in the parenchyma. Slight sclerosis and thickening of the cerebral arteries were observed, with hyalinization and calcification of the vessel walls. The section taken from the insula on the left side adjacent to the principal focus of destruction in the left basal ganglions revealed numerous small foci of encephalomalacia, some of which contained scattered gitter cells and polymorphonuclear leukocytes. The section taken from the principal focus of encephalomalacia revealed necrosis of the brain tissue with disintegration of the nerve and glial cells. Gitter cells were not found in this section. There was necrosis of the central part of a bundle of myelinated nerve fibers seen in the section taken from the right internal capsule. A few scattered leukemic cells were seen in this focus of necrotic tissue (fig. 4). Leukostasis was not observed in any of the sections studied.

Pathologic Diagnosis.—Myeloid leukemia with leukemic hyperplasia of the marrow; leukemic infiltrations of the spleen, lymph nodes, all mucous membranes, the kidneys, the testes and the adrenal glands; focal hemorrhages in the myocardium, the lungs and the subarachnoid space; infarcts of the spleen, the lungs and the kidneys; recent encephalomalacia involving the left internal capsule and the caudate nucleus; slight icterus; fibrous thickening of the leptomeninges.

CASE 8.—Chronic subleukemic myeloid leukemia in a 60 year old white man without neurologic signs or symptoms and no significant pathologic change in the brain.

The patient had noticed dizziness, weakness and attacks of fainting for four years prior to his admission to the hospital. These symptoms had increased in severity the month before admission, during which time he had noticed pallor of his skin and dyspnea and palpitation on slight physical exertion. During this period he had several attacks of nausea and vomiting. On admission the red blood cell count was 2,100,000, the hemoglobin content 6.6 Gm. and the platelet count 360,000. The white blood cell count was 1,500. The differential count revealed 16 myelocytes, 4 band forms, 16 segmented polymorphonuclear leukocytes and 64 lymphocytes. Sternal marrow obtained by aspiration showed 95 C myelocytes, 2 B myelocytes and 3 metamyelocytes. The heart was slightly enlarged to the left. After several transfusions the patient was discharged in an improved condition only to return to the hospital after a twenty-four day period with exacerbations of all his previous symptoms. On the second and final admission to the hospital, there was more severe anemia, the white blood cell count had risen to 29,400, and the differential blood cell count revealed 91 per cent B myelocytes. Signs and symptoms of uremia appeared one week before death,

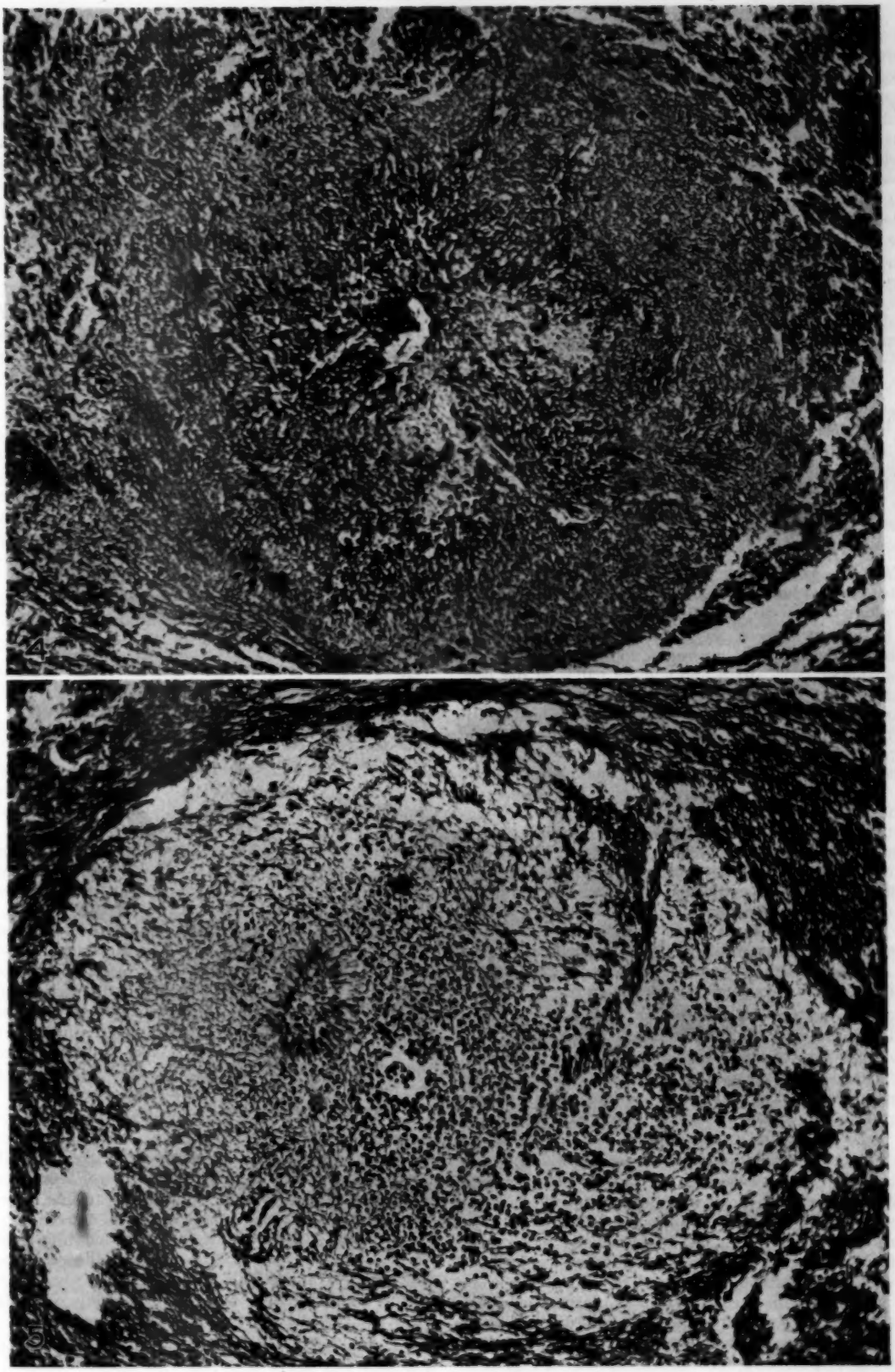

Fig. 4 (case 7 in the table).—Necrosis of the central part of a bundle of myelinated fibers in the right internal capsule. There are scattered leukemic cells and proliferating microglial cells in the peripheral part of the necrotic center. Hematoxylin and eosin stain; × 140.

Fig. 5 (case 4 in the table).—Focus of necrosis of the white matter containing scattered leukemic cells. Hematoxylin and eosin stain; × 140.

but at no time during the patient's illness were any neurologic signs or symptoms noted.

Postmortem Examination of the Brain.—The brain weighed 1,200 Gm. There was slight fibrous thickening of the leptomeninges, and small foci of subarachnoid hemorrhage were scattered over the convexities of the cerebral hemispheres. Serial sections through the brain revealed no gross pathologic change.

Histologic examination disclosed a moderate degree of leukostasis in all of the sections examined. The degree of leukostasis appeared to be more conspicuous in sections taken from the structures of the hindbrain. Accumulations of red blood cells in the perivascular spaces were observed in a section taken from the left basal ganglions. Two small foci of hemorrhage were encountered in the pons, one of which contained a group of leukemic cells.

Pathologic Diagnosis.—Myeloid leukemia with leukemic hyperplasia of the marrow; leukemic infiltrations of the brain, the liver, the spleen, the kidneys, the pancreas and the epicardium; hemorrhages in the brain, the subcutaneous tissues, the wall of the stomach; focal subarachnoid hemorrhage; bronchopneumonia of the upper lobe of the left lung, edema of the lungs; serofibrinous pleurisy on the right side; fibrous thickening of the leptomeninges.

CASE 9.—Acute monocytic leukemia in a 15 year old white boy without neurologic signs and only slight pathologic change in the brain.

The patient entered the hospital because of an unexplained elevation of temperature that was accompanied by weakness, tarry stools and marked pallor of the skin. These symptoms had been noted for three months. Examination revealed hypertrophic gums that bled easily. There was generalized enlargement of the superficial lymph nodes, and the spleen was palpable below the costal margin. The red blood cell count was 2,600,000 and the hemoglobin content 8.5 Gm. The differential count revealed 57 monoblasts, 23 lymphocytes, 7 segmented polymorphonuclear leukocytes, 6 stab forms, 2 juvenile forms and 5 myelocytes. Aspirated sternal marrow revealed a high percentage of monocytes, and the diagnosis of monocytic leukemia was made. Multiple abscesses of the subcutaneous tissues developed in the neck and over the thorax, the arms and the legs and the patient died after an illness known to have lasted nine and one-half months. At no time during the course of his illness were neurologic symptoms or signs noted.

Postmortem Examination of the Brain.—The weight of the brain was slightly increased, being 1,500 Gm. Two small foci of subarachnoid hemorrhage were seen on the medial surface of the right frontal lobe. Serial sections through the brain disclosed small foci of hemorrhage in the corpus callosum, the centrum ovale and the subcortical white matter in the left occipital lobe.

Histologic examination showed extensive leukostasis in the smaller vessels and small foci of leukemic cells in the white matter in all the sections taken. In most instances the foci of infiltration were surrounded by a small zone of necrotic brain tissue, and in most of the foci the necrotic tissue was larger than the focus of leukemic cells. Although scattered red blood cells were not infrequent in the perivascular spaces in these lesions, hemorrhage with extravasation of red blood cells into the tissue was noted only in the sections from the corpus callosum and the substantia nigra.

Pathologic Diagnosis.—Monocytic leukemia with leukemic hyperplasia of the marrow and lymph nodes; hypertrophy of the gingivae; leukemic infiltrations of the brain and the spleen; petechiae in the conjuctivas, the pleura, the pericardium, the peritoneum and the kidneys; focal hemorrhages in the subarachnoid space, the corpus callosum and the centrum ovale; fatty degeneration of the myocardium; multiple draining abscesses of the neck, the thorax, the arms and the legs; miliary abscesses of the myocardium; bilateral purulent mastoiditis with extradural loculations of pus; bronchopneumonia of all lobes of the lungs; fibrinopurulent pleurisy on the left side.

CASE 10.—Acute myeloid leukemia in a 2½ year old white girl, in whom hemiplegia developed three hours before death from a hemorrhage in the right centrum ovale.

The patient had suffered from nosebleeds for six months prior to her admission to the St. Louis Children's Hospital, and for two months before her entry it was noticed that she bruised easily. Physical examination on admission disclosed ecchymotic and purpuric spots in the skin. The liver and the spleen were palpable. The red cell count was 3,170,000 cells, the hemoglobin content 8.6 Gm. and the platelet count 45,000. The white blood cell count was 18,000, and the differential count showed 18 myeloblasts, 28 myelocytes, 10 juvenile forms, 12 segmented polymorphonuclear leukocytes and 32 lymphocytes. The bleeding time was prolonged, being thirty minutes. Three hours before death generalized convulsions developed, with labored respirations and spastic paralysis of the left side of the body. The right pupil was widely dilated, the left one was constricted and neither reacted to light. The total duration of the disease was eight months.

Postmortem Examination of the Brain.—The weight of the brain was moderately increased, being 1,110 Gm. There were scattered foci of subarachnoid hemorrhage over the right cerebral hemisphere and the cerebellum. Serial sections through the brain revealed a focus of hemorrhage measuring 4 by 5 cm. in the centrum ovale in the right parietotemporal region that extended medially to involve the lentiform nucleus.

Histologic study of a section taken from the region of hemorrhage in the right cerebral hemisphere revealed numerous small foci of hemorrhage in the white matter and in the cortical gray matter not involved in the principal area of hemorrhage. These foci of hemorrhage usually enclosed a small or medium-sized blood vessel. There was no apparent reaction in the brain tissue surrounding the hemorrhage. In sections from the left thalamus, a small hemorrhage was encountered beneath the ependyma in the wall of the third ventricle near the foramen of Monro. Only occasionally were small accumulations of leukemic cells associated with the foci of hemorrhage. Leukostasis in the blood vessels was not seen in any of the sections.

Pathologic Diagnosis.—Myeloid leukemia with leukemic hyperplasia of the marrow; leukemic infiltrations of the spleen and the brain; focal hemorrhages beneath the epicardium, in the myocardium, beneath the pleura, the peritoneum and the mucosa of the stomach and the colon, in the skin and in the lungs and the kidneys; petechiae in the skin, the conjunctivas and the mucosa of the urinary bladder; hemorrhage in the right cerebral hemisphere involving the lentiform nucleus and the subarachnoid space.

CASE 11.—Acute leukemia of an unclassified type in a 3 year old white girl who had no neurologic signs or symptoms and no pathologic changes in the brain.

Six months prior to her admission to the St. Louis Children's Hospital the child had an attack of pneumonia that was followed by severe anemia, and shortly before her admission to the hospital numerous ecchy-

moses of the skin developed. Physical examination on admission disclosed enlargement of the liver, the spleen and all the superficial lymph nodes. The red blood cell count was 3,800,000 and the hemoglobin content 9.7 Gm. The differential blood count revealed 95 blast cells and 5 segmented polymorphonuclear leukocytes. The platelet count was 25,000. After a temporary improvement following her admission, the patient's red blood cell count dropped to 2,000,000 and the white cell count to 1,500, the differential count being essentially unchanged. She died three months after her admission and nine months after the onset of her disease without ever having shown any neurologic signs or symptoms.

Postmortem Examination of the Brain.—The weight of the brain was slightly increased, being 1,100 Gm. There were numerous petechiae and ecchymoses in the dura mater. No pathologic change was noted on the external surfaces or in the serial sections made.

Histologic examination revealed no pathologic change in any of the sections taken.

Pathologic Diagnosis.—Leukemia of unclassified type with hemorrhage and fibrosis of the femoral and vertebral marrows; leukemic infiltration of the lymph nodes and the spleen; focal hemorrhages in the skin, the pleura, the subepicardial tissues, the kidneys, the peritoneum, the dura mater, the mucosa of the urinary bladder and the gastrointestinal tract; acute pyelonephritis; bronchopneumonia of all lobes of the lungs; serosanguinous pleurisy.

CASE 12.—Acute leukemia of an undetermined type in a 6½ year old white boy without neurologic symptoms or demonstrable pathologic changes in the brain.

A diagnosis of acute lymphoid leukemia had been made on a previous admission to the St. Louis Children's Hospital, two months before his final entry. There was good improvement in his condition following treatment, and for nearly two months after his discharge the child had been clinically well. One week prior to the second and final admission he became extremely weak, and it was noticed that he bruised easily. On final admission there were physical signs of consolidation in the right side of the chest and enlargement of the liver and the spleen. Roentgenologic studies of the chest confirmed the clinical diagnosis of pneumonia of the right lung. The red blood cell count was 2,100,000; the hemoglobin content was 5.9 Gm. There was a marked reduction in the number of platelets. The total white blood cell count was 10,500, and 96 per cent of the cells in the differential count were immature blast forms. Three days after admission he had a generalized convulsion that started on the left side and was controlled only with drop ether. He recovered from this attack without discernible residual neurologic signs. Two weeks later a second attack of generalized convulsions occurred that was controlled by intravenous injection of sodium amytal. However, the patient failed to regain consciousness and died in a few hours. The known duration of the clinical illness was four and one-half months.

Postmortem Examination of the Brain.—The weight of the brain was normal, being 1,320 Gm. No pathologic changes were seen on the external surfaces, nor were any abnormalities noted in the serial sections.

Histologic examination revealed only scattered red blood cells in the perivascular spaces surrounding the blood vessels in the section taken from the frontal lobe. Leukostasis was not noted in any of the sections.

Pathologic Diagnosis.—Leukemia of undeterminal type with leukemic hyperplasia of the marrow; leukemic infiltrations of the kidney; hemorrhages in the perirenal fat beneath the capsules of the kidneys; petechiae in the skin and in the mucosa of the gastrointestinal tract; bronchopneumonia of all lobes of the lungs; fibrinous pleurisy; hydropericardium; hydroperitoneum.

CASE 13.—Acute lymphoid leukemia in a 6½ year old white boy without pathologic changes in the brain or clinical neurologic signs.

Twenty-one months before his admission a small melanoma was removed from his skin, with radiation directed to the place of excision. Routine studies of the blood at that time disclosed a great elevation in the total white cell count, for which reason the child was referred to the Children's Hospital for complete hematologic study. Examination disclosed generalized enlargement of the lymph nodes and a palpable spleen. The red blood cell count was 2,300,000, and the hemoglobin content was 5.3 Gm. The white blood cell count was 36,000, and the differential count revealed that 90 per cent of the cells were nongranular mononuclear blast cells. The platelet count was 29,000. The patient's course in the hospital was complicated by an infection of the upper respiratory tract and bilateral otitis media. The administration of sulfadiazine and blood transfusions were ineffectual, and he died twenty-four days after his admission. At no time during the patient's illness were neurologic signs or symptoms noted.

Postmortem Examination of the Brain.—The brain weighed 1,350 Gm. No pathologic change was noted on the external surface or in the serial sections.

Histologic examination revealed scattered leukemic cells in the subarachnoid space in the sections taken from the cerebral hemisphere and the cerebellum. Red blood cells in moderate numbers were seen in the perivascular spaces in the section taken from the hypothalamus.

Pathologic Diagnosis.—Lymphoid leukemia; leukemic hyperplasia of the cervical and iliac lymph nodes and the marrow; leukemic infiltrations of the liver and the spleen; hemorrhages in the lungs, mesentery, testes, perirenal and periadrenal tissues and beneath the conjunctivas; petechiae in the dura mater, the skin, the mucosa of the renal pelves, the urinary bladder and the gastrointestinal tract; slight icterus; bronchopneumonia of all lobes of the lungs.

CASE 14.—Acute myeloid leukemia in a 12 year old white girl with no clinical signs of neurologic disease and no pathologic changes in the brain.

The patient was admitted to the St. Louis Children's Hospital with a two month history of bruising easily, great fatigability and enlargmenent of the spleen. Physical examination revealed slight enlargement of all the superficial lymph nodes, and the liver and the spleen were palpable. The red blood cell count was 2,500,000; the hemoglobin content, 7.3 Gm. The white blood cell count was 348,000, and the platelet count was 222,000. In the differential count 85 per cent of the cells were immature cells of the myeloid series. Radiation therapy and transfusions failed to improve the girl's condition, and she died one month after she entered the hospital and three and one-half months after the onset of her disease, without having shown any neurologic signs or symptoms.

Postmortem Examination of the Brain.—The brain weighed 1,260 Gm. No gross pathologic changes were noted on the external surfaces or in the serial sections.

Histologic examination revealed a slight degree of leukostasis in all sections taken but no other significant pathologic change. The roots of the third and

fifth nerves were examined, but no pathologic change was found.

Pathologic Diagnosis.—Myeloid leukemia; leukemic hyperplasia of the marrow; leukemic hepatomegaly and splenomegaly; infarcts of the spleen; petechiae in the skin, the kidneys and the mucosa of the esophagus, the stomach and the intestines; hemorrhagic bronchopneumonia of the lower lobes of the lungs; fibrinous pleurisy; serosanguinous hydrothorax.

CASE 15.—Chronic lymphoid leukemia in a 16 year old white girl without pathologic change in the brain or signs of neurologic disease.

The patient entered the Barnes Hospital with the history of a generalized enlargement of lymph nodes and an increase of lymphoid tissue of the pharynx that had been noted for two months. As a result of the enlargement of the lymphoid tissue in the pharynx her nose was almost completely obstructed. She also had lost hearing in the right ear, and for one week before entry she had fleeting headaches and dizziness. On admission physical examination revealed generalized enlargement of the lymph nodes and marked increase in the size of the tonsils and the lymphoid tissue over the posterior part of the pharynx. The liver and the spleen were slightly enlarged. During her stay in the hospital the red blood cell count fell steadily and reached a low of 1,800,000 shortly before death. The white blood cell count varied between 17,800 and 74,000, and the differential count showed from 59 to 90 per cent leukosarcoma cells. The platelet count was consistently low, the lowest count recorded being 6,000. Except for a single remission after the first month, following radiation therapy, the disease progressed steadily, and death followed a two day period of coma five months after the onset of the illness. At no time during the patient's illness were any neurologic signs or symptoms noted.

Postmortem Examination of the Brain.—The brain weighed 1,280 Gm. No pathologic change was noted on the external surfaces or in the serial sections.

Histologic examination revealed no pathologic change in any of the sections taken. The roots of the third nerve were examined but were free from pathologic change.

Pathologic Diagnosis.—Lymphoid leukemia involving all of the lymph nodes, the marrow, the spleen, the liver and the lungs; infarcts of the spleen; petechiae in the skin, all the mucous membranes, the peritoneum, the pleurae, the pericardium, the renal cortices and the pelves of the kidneys; hemorrhage in the fallopian tubes and lungs; bilateral hydrothorax; bronchopneumonia of all lobes of the lungs.

CASE 16.—Chronic lymphoid leukemia in a 78 year old white woman without pathologic changes in the brain or symptoms of neurologic disease.

The patient entered the Barnes Hospital with the history that the lymph nodes in her neck had been enlarged for two years and that during the past six months she had noticed enlargement of the axillary lymph nodes. Recently she noticed unusual fatigability on small exertion. On admission to the hospital physical examination revealed hard, fixed masses in the neck and the axillas. Roentgenologic studies of the chest disclosed great enlargement of the mediastinal shadow. The red blood cell count was 2,800,000, and there was 9.0 Gm. of hemoglobin per hundred cubic centimeters of blood. The white blood cell count was 98,000, and 97 per cent of the cells were lymphoblasts, as determined by the differential count. Radiation therapy was tolerated well but did not alter the course of the disease. On the eighth day of hospitalization the patient became irrational and vomited occasionally. She died the same day after a blood transfusion, never having shown any neurologic signs or symptoms. The known duration of the disease was two years.

Postmortem Examination of the Brain.—The brain weighed 1,270 Gm. There was moderate sclerosis of the cerebral arteries, but no other lesions were noted either in the routine external examination or in the serial sections made. Blocks of tissue were taken at the time of necropsy and fixed in 80 per cent alcohol.

The sections were stained with thionine. In the histologic examination a focus of leukemic infiltration was seen in the section taken from the medulla oblongata. There was no leukostasis in any of the sections examined. Chromatolysis of a high degree was present in most of the nerve cells in the sections taken from the cortex. Occasionally a nerve cell appeared shrunken, and the nucleus was eccentrically placed and pyknotic.

Pathologic Diagnosis.—Lymphoid leukemia; leukemic hyperplasia of the axillary, mediastinal, periaortic, portal hepatic, peripancreatic, mesenteric and inguinal lymph nodes and the marrow; leukemic infiltrations of the brain, the liver, the spleen, the kidneys and the lungs; bilateral hydrothorax; focal hemorrhages in the pleura of the left lung and in the mucosa of the urinary bladder; moderate sclerosis of the cerebral arteries.

CASE 17.—Acute myeloid leukemia in a 16 year old white boy without pathologic changes in the brain and no neurologic symptoms.

On entering the Barnes Hospital the patient gave the history of having had a sore throat, a pleuritic type of pain, migratory arthritis and a slight elevation of body temperature for the preceding five weeks. Physical examination revealed enlargement of the cervical and inguinal lymph nodes and small hemorrhages in the ocular fundi and in the buccal mucosa. The red blood cell count was 2,400,000, the hemoglobin content 7.1 Gm. and the white blood cell count 98,700. The differential count revealed 8 myeloblasts, 78 myelocytes, 4 metamyelocytes, 4 segmented polymorphonuclear leukocytes, 1 lymphocyte and 5 monocytes. The patient was in the hospital but three days before his death, during which time he frequently vomited black liquid material and passed blood per rectum. A few hours before death he became irrational, then lapsed into coma. At no time during the illness was there noted any neurologic sign or symptom. The duration of disease was five weeks.

Postmortem Examination of the Brain.—The weight of the brain was moderately increased, being 1,740 Gm. Except for moderate flattening of the cerebral convolutions, no gross pathologic change was noted in the external examination or in the serial sections.

Histologic examination revealed leukostasis of the blood vessels in all sections taken. Leukemic cells were seen in the perivascular spaces in a few of the vessels in the sections from the thalamus, the pons and the medulla.

Pathologic Diagnosis.—Myeloid leukemia; leukemic hyperplasia of the lymph nodes and the marrow; leukemic infiltrations of the liver and the spleen; petechiae in the peritoneum and the pleura, beneath the endocardium of the left ventricle, in the cortices of the kidneys and in the mucosa of the esophagus and the stomach.

CASE 18.—Chronic myeloid leukemia in a 24 year old white man with significant pathologic changes

occurring in the brain although the only neurologic symptoms were disturbance of vision and papilledema.

The patient was known to have had chronic myeloid leukemia for ten years. His disease was well controlled by means of radiation therapy and solution of potassium arsenite U. S. P. until three weeks before his death, at which time there developed headaches, shortness of breath and blurring of vision that was most severe in the left eye. Ocular examination on admission to the hospital showed marked constriction of peripheral vision in the left eye, bilateral papilledema and many retinal hemorrhages in the ocular fundi. The spleen and the liver were greatly enlarged. The red blood cell count was 2,000,000, and the hemoglobin content was 7.5 Gm. The white blood cell count was 350,000, and the differential blood count revealed 46 myelocytes, 26 juvenile forms and 28 segmented polymorphonuclear leukocytes. The platelets were too few to count. The patient became stuporous and died the second day that he was in the hospital. At no time prior to the last three weeks of his illness were neurologic signs or symptoms noted.

Postmortem Examination of the Brain.—The weight of the brain was moderately increased, being 1,750 Gm. The right parieto-occipital region was bulging and firm to palpation. Serial sections through the brain revealed moderately large foci of leukemic infiltration and hemorrhage throughout the cerebral hemispheres. The largest foci were found in the right frontal, left temporal, right parietal and right occipital lobes. The greater part of the right occipital lobe was destroyed by the hemorrhage and infiltration; the largest focus in this part of the brain measured 5 cm. in diameter (fig. 1). Other lesions measuring from 2 to 3 cm. in diameter were noted in the parts of the brain mentioned. In addition to these larger foci there were numerous smaller lesions of similar character scattered in all parts of the brain, measuring from 1 to 2 mm. in diameter. The pathologic changes were almost entirely limited to the white matter, only the largest foci extending into the cortical gray matter.

The routine sections taken from the brain were stained with thionine for the demonstration of Nissl substance, as well as with the routinely used hematoxylin and eosin. Histologic study revealed marked leukostasis of the blood vessels in all the sections taken (fig. 7). In the sections taken from the foci of hemorrhage and leukemic infiltration there were small discretely outlined collections of leukemic cells in the brain tissue, usually surrounded by a small peripheral zone of necrotic brain tissue. The blood vessels in some of these lesions were necrotic. In other instances, however, normal vessels were seen, even within the large foci of leukemic infiltration. Hemorrhage associated with the focus of infiltration was usually observed, but occasionally large foci of infiltration composed of many coalescing smaller foci of infiltration were observed without hemorrhage (fig. 3). Chromatolysis of a severe degree was noted in the nerve cells in all of the sections taken from the cerebral cortex. Sections taken from the roots of the second, fourth, fifth, sixth, seventh, eighth, tenth and twelfth nerves showed no pathologic change except leukostasis of the blood vessels.

Pathologic Diagnosis.—Myeloid leukemia; leukemic hyperplasia of the marrow; leukemic splenomegaly and hepatomegaly; extensive leukemic infiltrations with hemorrhage in the brain; leukemic infiltrations of the kidneys and the lungs; multiple small hemorrhages in the myocardium and beneath the epicardium; cirrhosis of the liver; bronchopneumonia of all lobes of the lungs.

CASE 19.—Leukosarcoma in a 54 year old white woman with significant pathologic changes in the brain but no neurologic signs.

The patient had complained of malaise for five months before entering the hospital, during which time her body temperature was elevated and the spleen enlarged. It was thought that she had malaria, and her symptoms were partly alleviated by antimalarial therapy. The symptoms recurred, and four weeks before admission it was noted that the cervical lymph nodes were enlarged. On entry there was enlargement of the liver and the spleen with a white blood cell count of 270,000, 98 per cent of the cells being leukosarcoma cells. The thrombocyte count was 25,000, and hemorrhages were noted in the skin, the ocular fundi and the buccal mucosa. She was treated with radioactive phosphorus, and the white blood cell count dropped to 3,650, but there was no significant change in the differential count. The red blood cell count was severely reduced, to 630,000, presumably as a result of the therapy. In the terminal stage of the disease she was frequently nauseated and vomited often. Following a period of coma the patient died, the known duration of her disease being nine months. At no time during the course of her illness did she have any neurologic signs or symptoms.

Postmortem Examination of the Brain.—The brain weighed 1,280 Gm. There was a thin layer of clotted blood beneath the under surface of the dura mater that was not attached and showed no evidence of organization. Focal hemorrhages were noted in the subarachnoid space over the posterior and lateral aspects of the cerebellar hemispheres, the inferior surface of the left frontal lobe and the pons. Serial sections through the brain revealed groups of focal hemorrhages, averaging approximately 1 mm. in diameter, in the anterior part of the corpus callosum, the basal ganglions, the roof of the inferior horn of the right lateral ventricle, the tegmentum of the mesencephalon, the wall of the fourth ventricle, the medulla oblongata and in the folia and the white matter of the cerebellum. A focus of light red discoloration of the nervous tissue, 5 mm. in diameter, was noted in the anterior part of the left cerebral peduncle.

Histologic examination of a section taken from the blood clot beneath the dura mater revealed well preserved red blood cells and scattered white blood cells, the greater number of which resembled lymphocytes. Sections taken from the foci of hemorrhage noted in the gross examination showed accumulations of red blood cells lying free in the tissue without evidence of leukemic infiltration. Leukostasis was present in all of the sections. In a section taken from the corpus callosum there were many small scattered hemorrhages in the tissue without evidence of leukemic infiltration (fig. 6). Many small foci of infiltration with leukemic cells that frequently were associated with hemorrhage were seen in many of the sections.

Pathologic Diagnosis.—Lymphosarcoma involving the stomach, the jejunum, the cecum, the ascending colon and the lower lobe of the right lung; lymphatic leukemia; leukemic hyperplasia of the marrow and lymph nodes; leukemic splenomegaly; leukemic infiltrations of the brain; extensive hemorrhage in the subdural space; moderate subarachnoid hemorrhage; focal hemorrhages in the brain, the skin, the pleura, the pericardium, the myocardium, the endocardium, the esophagus, the stomach and the mucosa of the urinary

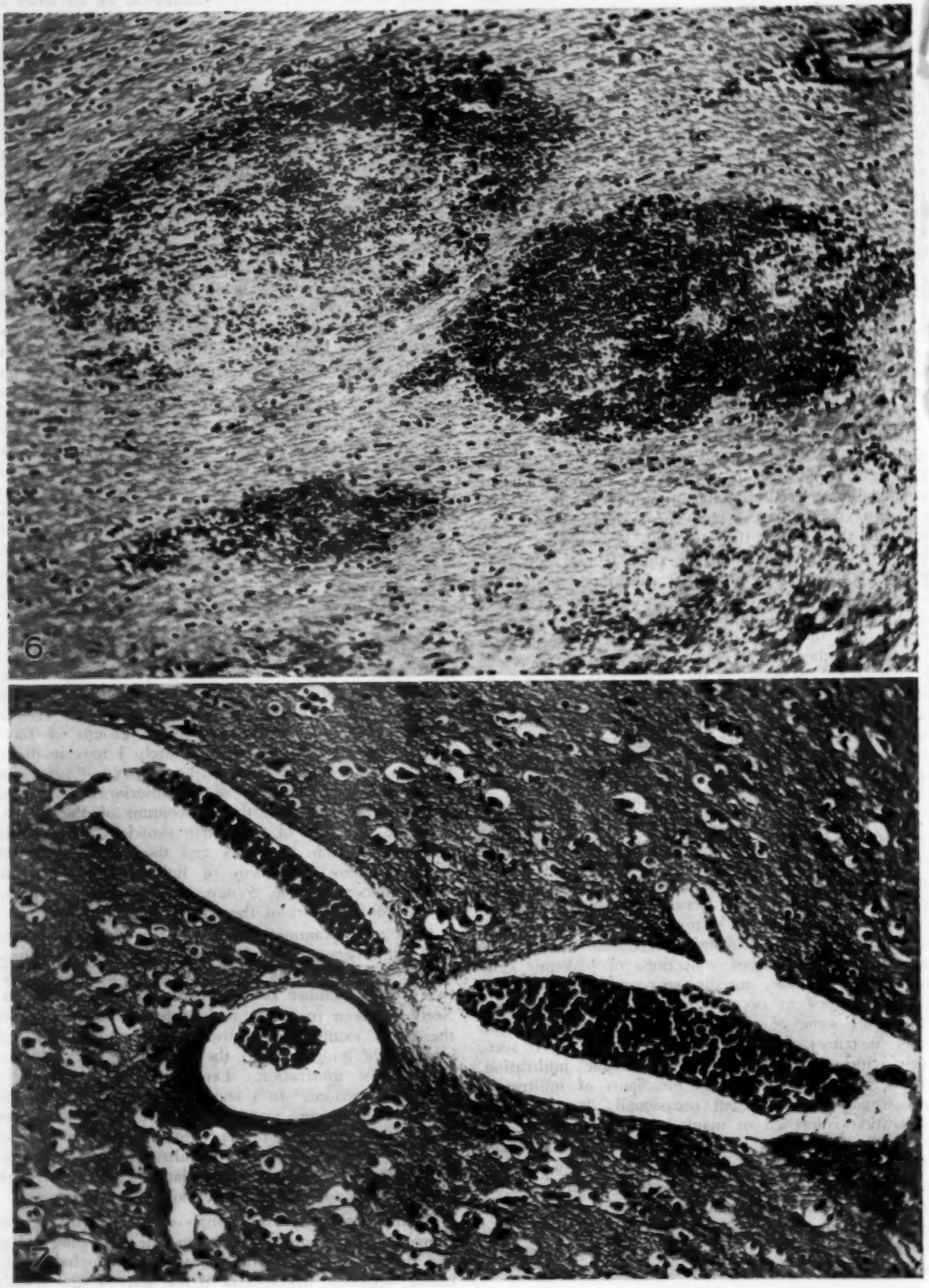

Fig. 6 (case 19 in the table).—Foci of hemorrhage in the corpus callosum without infiltrations of leukemic cells. There is necrosis of the white matter within the focus of hemorrhage in the upper left part of the field, evidenced by the pale staining of the tissue. Hematoxylin and eosin stain; × 140.

Fig. 7 (case 18 in the table).—Leukostasis: Three small venules are filled with leukemic cells without red blood cells. Hematoxylin and eosin stain; × 180.

bladder; bronchopneumonia in all lobes of the lungs; fatty metamorphosis of the liver; fatty degeneration of the myocardium.

CASE 20.—Chronic myeloid leukemia in a 37 year old white woman without demonstrable pathologic change in the brain but with a few neurologic signs.

The patient was known to have had chronic myeloid leukemia for a year and a half and had been treated with radioactive phosphorus. Her principal symptoms before her final admission to the hospital were great fatigability and pain in her left side, presumably from an enlarged spleen. Examination revealed marked enlargement of the liver and the spleen. There was moderate anemia, the red blood cell count being 3,700,000 and the hemoglobin content 10 Gm. The white blood cell count was 216,000, and a differential count revealed 3 basophilic cells, 3 juvenile forms, 4 stab forms, 29 segmented polymorphonuclear leukocytes, 60 myelocytes and 1 lymphocyte. There were 51,000 platelets. In spite of continued treatment with radioactive phosphorus and radiant energy, the patient died two weeks after her admission to the hospital. Thirty minutes before death the deep reflexes could not be elicited and the patient became obtunded. During this period there were jerky movements of the right arm and leg, the left side of the face was "drawn up," and the eyes, without nystagmus, were turned toward the left.

Postmortem Examination of the Brain.—The brain weighed 1,140 Gm. There were no gross pathologic changes on the external surfaces or in the serial sections made.

Histologic study of the brain revealed only a slight amount of leukostasis in a few of the sections taken.

Pathologic Diagnosis.—Myeloid leukemia; leukemic hyperplasia of the marrow; leukemic splenomegaly and hepatomegaly; old and recent infarcts of the spleen; congestion and edema of the lungs; focal atelectasis of the lungs.

LITERATURE

We have collected from the literature 47 cases of leukemia with adequate pathologic study of the brain to be of value in our study. Our criterion for acceptance of a case was an adequate description of the cerebral lesions as well as sufficient information on the clinical course of the disease. Sufficient clinical data for our purposes were usually available if the anatomic study of the case was sufficiently detailed. Cases reported with data pertinent only to lesions occurring in the spinal cord and the cranial nerves were excluded, as they were of no comparative value in this study. Cases in which there were significant lesions of the cranial nerves were too infrequent to afford opportunity for collective study. The cases included in the studies of Ebstein,[7] Grosz,[8] Marchand,[9] Oeller,[10] Bass[11] and Recklinghausen,[12] the second case in the reports of Benda,[13] Rosenkranz,[14] Singer and Nevinny,[15] cases 4 and 7 in the report of Viets and Hunter[16] and cases 1 and 2 in the reports of Hellich[4] failed to meet our criteria. The cases studied by Eisenlohr,[17] May,[18] Alt and Pineles,[19] Hellgardt,[20] Trömmer and Wohlwill,[21] Spangenberg,[22] Löwy,[23] Garvey and Lawrence,[24] Murphy and Brody[25] and cases 4, 8 and 11 reported by Diamond[2] were described mainly with respect to lesions of the cranial nerves and for this reason were not included. The collected cases, including the 20 reported in this paper, are listed in the accompanying table.

STUDY OF THE COLLECTED CASES

Not all of the cases collected from the literature can be regarded as selected at random, since in most instances the reason for publication was that there had been unusual clinical neurologic signs and symptoms indicating involvement of the central nervous system. However, all of the cases in Diamond's series (cases 34, 35, 36, 37, 38, 39, 40, 41, 42, 43 and 44 in the table) and the 20 cases studied by us were selected at random, so that this group of 31 cases can be considered as unselected cases of leukemia. For certain points of analysis—for example, the determining of the incidence of clinical neurologic signs and symptoms in cases of leukemia—only these 31 cases are significant, and in all subsequent discussions they will be referred to as the random series. The general clinical and pathologic features of leukemia as it affects the brain can be determined

7. Ebstein, W.: Deutsches Arch. f. klin. Med. **44**: 343, 1888-1889.

8. Grosz, K., quoted by Strümpell: Wien. klin. Wchnschr. **23**:603, 1910.

9. Marchand, F.: München. med. Wchnschr. **58**: 1215, 1911.

10. Oeller, H.: Deutsche Ztschr. f. Nervenh. **47**: 504, 1913.

11. Bass, M. H.: Am. J. M. Sc. **162**:647, 1921.

12. von Recklinghausen, F.: Virchows Arch. f. path. Anat. **30**:370, 1864.

13. Benda, C.: Berl. klin. Wchnschr. **35**:228, 1898.

14. Rosenkranz, G.: Frankfurt. Ztschr. f. Path. **35**: 359, 1927.

15. Singer, L., and Nevinny, H.: Virchows Arch. f. path. Anat. **268**:576, 1928.

16. Viets, H. R., and Hunter, F. T.: Arch. Neurol. & Psychiat. **29**:1246, 1933.

17. Eisenlohr, C.: Virchows Arch. f. path. Anat. **73**:56, 1878.

18. May, F.: Aerztl. Int.-Bl. München **31**:343, 1884.

19. Alt, F., and Pineles, F.: Wien. klin. Wchnschr. **21**:849, 1896.

20. Hellgardt, H. H.: Diplegia facialis bei Leukämie, Inaug. Dissert., Königsberg i. Pr., R. Leupold, 1917.

21. Trömner, E., and Wohlwill, F.: Deutsche Ztschr. f. Nervenh. **100**:233, 1927.

22. Spangenberg, J. J.: Prensa méd. argent. **15**:329, 1928.

23. Löwy, K.: Monatschr. f. Ohrenh. **66**:1134, 1932.

24. Garvey, P. H., and Lawrence, J. S.: J. A. M. A. **101**:1941, 1933.

25. Murphy, J. P., and Brody, B. S.: J. A. M. A. **115**:1544, 1940.

No.	Author	Age	Sex	Pathologic Changes in Brain and Meninges				
				Hemiplegia	Hemorrhage or Leukemic Infiltration of Subarachnoid or Subdural Space	Hemorrhage in Brain	Leukemic Infiltration in Brain	Cerebral Hemorrhage Responsible for Death
1	Reincke, J.: Virchows Arch. f. path. Anat. **51**: 391, 1870	22	M	0	?	+	0	?
2	Lauenstein, O.: Deutsches Arch. f. klin. Med. **18**: 120, 1876	50	M	+	0	+	+	+
3	Friedländer [26]	30	M	0	0	+	+	+
4	Fränkel [26]	18	M	+	+	+	?	+
5	Bramwell, B.: Brit. M. J. **1**: 1098, 1886	40	M	0	0	+	+	+
6	Guttmann, P.: Berl. klin. Wchnschr. **28**: 1100, 1891	10	M	+	0	+	?	+
7	Kast [27]	50	M	0	0	0	0	0
8	Benda,[13] case 1	15	F	?	0	+	+	+
9	Strauss, H.: Arch. f. Kinderh. **30**: 272, 1900	1	M	0	+	?	?	?
10	Spitz, R.: Deutsche Ztschr. f. Nervenh. **19**: 467, 1901	47	F	0	0	0	+	0
	Veszprémi [5]							
11	Case 1	43	F	0	0	0	+	0
12	Case 2	2	F	0	0	+	+	0
13	Case 3	25	F	0	0	+	+	0
14	Flessinger and Marie [28]	34	M	0	+	+	+	0
15	Taple and Cassar [24]	24	M	+	0	+	+	+
16	Munro [37]	17	M	0	+	0	+	0
17	Kwasniewski: Deutsches Arch. f. klin. Med. **145**: 83, 1924, case 2	47	F	0	+	?	?	?
18	Fried [31]	25	M	0	0	+	+	+
19	Rocchi [39]	65	M	0	0	+	+	+
20	Rosenkranz,[14] case 1	16	M	0	0	+	+	?
21	Singer and Nevinny [15]	31	M	0	+	+	+	0
22	Lengsfield [35]	9	M	0	+	0	0	0
23	Winkler [38]	24	F	0	+	+	0	0
24	Costa, A.: Sperimentale **86**: xxvii-xxxii, 1932	10	M	0	0	+	+	+
25	Howell and Gough[29]	11	M	0	0	+	+	0
	Hellich [4]							
26	Case 3	43	M	+	0	+	+	+
27	Case 4	32	F	+	+	+	?	+
28	Case 5	46	F	0	?	+	+	0
29	Hawksley, J. C.: Arch. Dis. Childhood **7**: 29, 1932	10	F	+	0	+	+	+
	Hamburger [3]							
30	Case 3	23	M	0	+	+	+	+
31	Case 4	36	M	0	0	+	+	?
32	Case 5	51	F	0	+	+	0	+
33	Viets and Hunter,[10] case 5	57	M	+	+	+	0	+
	Diamond [2]							
34	Case 1	74	M	0	0	0	0	0
35	Case 2	48	M	0	+	+	+	0
36	Case 3	39	M	0	+	+	0	0
37	Case 5	48	M	0	+	0	+	0
38	Case 6	36	M	0	+	+	+	+
39	Case 7	64	M	0	+	+	+	+
40	Case 9	4	M	0	+	+	+	0
41	Case 10	13	M	0	+	+	+	+
42	Case 12	66	M	0	0	+	+	0
43	Case 13	68	M	0	+	0	0	0
44	Case 14	28	M	0	+	+	+	?
45	Tarro [32]	16	M	?	0	+	+	+
46	Schwab and Weiss [1]	24	M	0	+	0	+	0
47	Massachusetts General Hospital, Cabot Case 25492, New England J. Med. **221**: 906, 1939	24	F	0	+	+	+	+
	Leidler and Russell							
48	Case 1	60	F	0	+	+	0	0
49	Case 2	14	F	0	+	+	+	+
50	Case 3	32	M	0	+	+	+	0
51	Case 4	38	F	0	+	+	+	+
52	Case 5	5	M	0	+	0	0	0
53	Case 6	49	F	+	0	+	0	+
54	Case 7	34	M	+	+	+	0	+
55	Case 8	60	M	0	+	+	+	0
56	Case 9	15	M	0	+	+	+	0
57	Case 10	2	F	+	+	+	+	+
58	Case 11	3	F	0	+	0	0	0
59	Case 12	6	M	0	0	0	0	0
60	Case 13	6	M	0	+	0	0	0
61	Case 14	12	F	0	0	0	0	0
62	Case 15	16	F	0	0	0	0	0
63	Case 16	78	F	0	0	0	+	0
64	Case 17	16	M	0	0	0	0	0
65	Case 18	24	M	0	0	+	+	+
66	Case 19	54	F	0	+	+	+	?
67	Case 20	37	F	0	0	0	0	0

by analysis and study of this group of 67 cases (see table) under the following heads.

Age and Sex.—In the collected cases all age groups are well represented. The youngest patient was 13 months and the oldest 78 years old. The ages of the patients were well distributed by decades, there being 12 each in the first and second decades, 11 each in the third and fourth decades, 9 in the fifth decade, 6 in the sixth decade, 4 in the seventh decade and 2 in the eighth decade. There was a 2 to 1 preponderance of male patients, there being 44 male patients and only 23 female patients.

Types of Leukemia.—There were 19 cases of acute myeloid leukemia, 13 of chronic myeloid leukemia, 9 of acute lymphoid leukemia, 8 of chronic lymphoid leukemia, 3 of monocytic leukemia, 1 of leukosarcoma and 15 of leukemia of an unclassified type. The percentage incidence of hemiplegia, fatal cerebral hemorrhage, hemorrhagic and leukemic infiltrations of the brain, and leukostasis has been computed for each type of leukemia to determine whether any one type involved the brain more frequently than the others. It was found that there was no significant difference between the types of leukemia in percentage incidence of hemiplegia, fatal cerebral hemorrhage and leukostasis. However, the incidence of hemorrhagic and leukemic infiltration of the brain was 20 per cent higher in the chronic than in the acute forms of lymphoid and myeloid leukemia. Because the number of cases of each type of leukemia is small, a difference of 20 per cent in a percentage incidence for any one type can only be taken as a suggestive result.

Neurologic Signs and Symptoms.—Neurologic signs and symptoms attributable to the leukemic disease were present in 34 of the 67 cases. Such signs or symptoms were present in 11 of the 31 cases in the random series. The duration of the neurologic signs and symptoms before death was known in 24 of the cases and varied in these cases from thirty minutes to eighteen months. In 7 of the cases neurologic symptoms were present only during the last day of life; in 5, for longer than one day but less than one week; in 7, for more than one week but less than one month; in 3, for more than one month but less than six months; in 2, for more than six months. The cases reported by Friedländer,[26] Kast,[27] Winkler,[28] Howell and Gough[29] and Schwab and Weiss[1] are deserving of note because the neurologic symptoms in these cases were the first symptoms of the leukemic disease. The duration of the neurologic symptoms before death in this group was generally greater since it varied from forty days to eighteen months.

The signs and symptoms of increased intracranial pressure were present in only a small number of the cases, although there were significant pathologic changes in the brain resulting from the leukemic disease in 92 per cent. Headache was present in 19, vomiting in 12 and papilledema in only 5. Headache, vomiting and papilledema occurred together in only 2 cases (22 and 23 in the table).

Hemiplegia was observed in 11 of the 65 cases in which the clinical history given was clear as to whether this complication was or was not present, and in all of the cases of hemiplegia there was an intracranial hemorrhage that was the immediate cause of death. However, hemorrhage of a proportion to account for death occurred in 27 of the 67 cases but was unaccompanied by hemiplegia in 14.

Paralysis of one or more cranial nerves was noted in 12 of the cases and was known not to be present in 53. Disturbances of vision and of the vestibular and auditory systems, as well as convulsive manifestations, were but infrequently encountered, each of these neurologic manifestations being noted in only 10 per cent or less of the cases. The most frequently recorded neurologic sign was a change in the deep or the superficial reflexes, there being 18 cases of leukemia with reported changes in reflexes.

Pathologic Changes in the Brain.—In the descriptive consideration of the gross and microscopic lesions occurring in the brain those observed in the 20 cases studied by us will be used.

(*a*) Changes in the Dura Mater and the Leptomeninges: Some pathologic alterations were noted in these structures in 35 of the 65 cases in which mention was made of their examination. Hemorrhages with or without infiltrations of leukemic cells occurred in the dura mater and in the subdural and subarachnoid spaces. Trömmer and Wohlwill[21] noted that the foci of cellular infiltration in the leptomeninges were frequently continuous with infiltrations extending into the cranial nerves. In many instances the leukemic infiltrations in the subarachnoid space demonstrated a perivascular pattern, but occasionally the cells were diffusely scattered in this space. Hemorrhage without an

26. Friedländer, C.: Virchows Arch. f. path. Anat. **78**:362, 1879.

27. Kast, A.: Ztschr. f. klin. Med. **28**:79, 1895.

28. Winkler, W.: Ztschr. f. d. ges. Neurol. u. Psychiat. **137**:385, 1931.

29. Howell, A., and Gough, J.: Lancet **1**:723, 1932.

accompanying infiltration of leukemic cells was occasionally seen.

(*b*) Hemorrhage in the Brain: Grossly visible foci of hemorrhage were observed in the brain in 47 of the 65 cases in which specific mention was made of an examination for this occurrence, and in 36 of the brains histologic study showed that the foci of hemorrhage were accompanied by infiltrations of leukemic cells. In the random series there were 19 cases with grossly visible foci of hemorrhage. Diffuse hemorrhage in the white matter without accompanying infiltration of leukemic cells was occasionally seen in brains where significant leukemic infiltration accompanied hemorrhage in other parts of the brain. In certain instances the hemorrhage caused necrosis of the white matter (fig. 6). In 7 of the brains the hemorrhage was unaccompanied by leukemic infiltration.

Hemorrhage in the brain of a proportion to be the immediate cause of death was a frequent complication, being present in 27 of the 60 cases in which adequate information pertinent to this finding was available. In the random series the incidence of cerebral hemorrhage causing death was slightly lower, this complication being present in only 9 of the 31 cases.

(*c*) Infiltrations of Leukemic Cells in the Brain: Infiltrations of leukemic cells in the parenchyma of the brain, excluding those in the perivascular spaces, were encountered in 42 of the 67 cases; such infiltrations were statedly not present in 20 cases. In 24 of the 42 cases in which they occurred the foci of infiltration measured 5 mm. in diameter or less. In 11 of the cases the foci were greater than 5 mm. but not over 2 cm., and in 7 cases the foci measured more than 2 cm. in diameter. Grossly the foci of infiltration appeared hemorrhagic and were moderately soft in consistence. The smallest ones consisted of densely packed leukemic cells and each was usually surrounded by a small zone of changed white matter (fig. 2). Lesions of this type occurred singly or in groups. A direct relation between the nodule and a blood vessel was seen in many instances. The larger, more extensive foci of infiltration apparently resulted from coalescence of many of the smaller ones. An example of the larger focus of infiltration (figs. 1 and 3) was seen in the lesion in the occipital lobe in our case 18 (case 65 in the table). Leukemic infiltrations of the perivascular spaces were always present when there was infiltration of the brain parenchyma with leukemic cells, and in 5 of our cases (1, 5, 6, 12 and 17 in the table) this was the only place in the brain where leukemic cells were observed. We have been unable to attach great significance to the mere presence of leukemic cells in the perivascular spaces, since it is rarely observed apart from infiltrations of leukemic cells in the brain parenchyma and therefore occurring alone it would be of doubtful significance in the diagnosis of leukemia. Moreover, the studies of Konschegg and Ott [30] have shown that accumulations of mononuclear cells in the perivascular spaces in the brain are not infrequently observed in patients with no known disease of the nervous system if the brain is thoroughly examined.

(*d*) Leukostasis: This is the phenomenon of engorgement of blood vessels with white blood cells in the complete or almost complete absence of erythrocytes (fig. 7) and is seen in other somatic tissues besides the brain. Leukostasis was present in 41 of the cases and stated not to be present in 10. In the random series, leukostasis was noted in the brain in 24 of the 31 cases. The importance of leukostasis in the development of the leukemic infiltrations of the brain is suggested from the fact that it was absent in only 3 of the 33 brains having infiltrations with leukemic cells in which that change was searched for. In these 3 brains the foci of infiltration were small, the largest being not more than 5 mm. in diameter. Leukostasis was present in 9 brains in which infiltrations of the parenchyma with leukemic cells were not observed.

(*e*) Changes in the Nerve Cells: A large number of pathologic changes have been described in the nerve cells of the brain in leukemia, including chromatolysis, reticulation of the cytoplasm, eccentricity of the nucleus, shrinkage of the cell, fatty degeneration, neuronophagia and necrosis and liquefaction of the cell. Diamond,[2] Kast,[27] Fried [31] and Howell and Gough [29] have described significant pathologic changes in the nerve cells in all of their cases and intimated that these changes were the direct result of the leukemic disease. Winkler [28] observed foci of disrupted nerve fibers in which many of the nerve cells had disappeared, and Hamburger [3] observed necrosis and destruction of nerve cells surrounding foci of hemorrhage. We have observed many of the changes described by these authors surrounding foci of infiltration of leukemic cells and hemorrhage, but in our opinion these changes resulted from the effect of the hemorrhage and the infiltration of cells and not from any specific effect of the leukemic disease acting directly on the nerve cells. More-

30. Konschegg, T., and Ott, E.: Virchows Arch. f. path. Anat. **306**:538, 1940.

31. Fried, B. M.: Arch. Path. **2**:23, 1926.

over, in most of our cases the examination of a sufficient number of sections revealed reticulation of the cytoplasm, eccentricity of the nucleus and shrinkage of an occasional cell in a large number of instances. These pathologic changes were best seen in the 2 cases in which sections stained for Nissl substance showed chromatolysis of an advanced degree in the nerve cells of the cerebral cortex. We found no evidence to indicate that any of these changes were the direct result of leukemic disease affecting the nerve cells, since all of the changes could be easily accounted for as secondary to the physical alteration of the brain caused by the hemorrhage and leukemic infiltrations or by the agonal state.

(*f*) Changes in the White Matter: In 46 of the cases mention of examination of the white matter was made, but in only 10 of these was there description of significant pathologic change. The changes observed consisted of swelling of the myelin sheath and axon, vacuolation of the myelin in the myelin sheath and disruption of the arrangement of the fibers (fig. 4), in most instances without demonstrable reaction of the neuroglial cells. In our series there were 3 cases (4, 7 and 19 in the table) in which foci of altered staining of the myelin were observed. These foci frequently contained scattered leukemic cells (fig. 5) and occasionally were surrounded by small nodules of proliferating microglial cells. The foci of altered staining of the white matter were believed to be the result of disruption of the fibers produced by hemorrhage or focal leukemic infiltration in the tissue at some distance from the observed change in the white matter.

(*g*) Changes in the Neuroglia: In 17 of the 46 cases in which the neuroglia was described, some type of pathologic change was reported. The changes observed consisted of proliferation of astrocytes and microglial cells surrounding the focal lesions with formation of gitter cells. In rare instances focal accumulations of glial cells were seen throughout the brain without a demonstrable lesion in the parenchyma. In our series of cases increased numbers of glial cells with formation of gitter cells were seen usually surrounding foci of leukemic infiltration and hemorrhage, particularly if these had been present for some time. Slight astrogliosis was noted around the older lesions. We believe that these changes were secondary to injury produced in the brain by hemorrhage and leukemic infiltration and were not the result of the leukemic disease.

(*h*) Changes in the Blood Vessels: Several types of pathologic change occurring in the cerebral blood vessels in leukemia have been described, and of the 59 cases in which pathologic examination of the blood vessels was mentioned some change was noted in 19. Diamond [2] has described the most extensive pathologic changes, for in all of his cases he observed swelling, hypertrophy and proliferation of the endothelial cells, proliferation of adventitial cells, proliferation of capillaries and occasionally hyalinization of the vessel wall. Fried,[31] Singer and Nevinny [16] and Tarro [32] described similar but less extensive changes. Fiessinger and Marie [33] have suggested that possibly the hemorrhages occurring in the brain in leukemia are caused by some and as yet unidentified proteolytic enzyme liberated from the leukemic cells which digests the capillary walls. Tapie and Cassar [34] described rupture of capillaries in regions of the brain showing leukostasis, and Rosenkranz [14] described destruction of the elastic tissue in the walls of blood vessels occurring near foci of hemorrhage. We have not observed these pathologic changes in our cases. A significant pathologic change was encountered by us in only 1 case (18 in the table), this being necrosis of the wall of a blood vessel within a locus of infiltrating leukemic cells. In most of the cases in our series there was remarkable preservation of the blood vessels of the brain, even within the foci of leukemic infiltration.

(*i*) Involvement of Cranial Nerves: In spite of the importance of cranial nerves in neurologic diagnosis, morphologic study of these structures in cases of leukemia has been infrequent. The pathologic changes that have been described in the cranial nerves were similar to the changes noted in the brain, namely, leukemic infiltrations, usually accompanied by hemorrhage. Of the 17 cases with adequate study of the cranial nerves, pathologic changes were described in the seventh nerve in 13, in the second nerve in 6, in the eighth nerve in 5, in the third nerve in 3, in the fifth and tenth nerves, respectively, in 2, and in the fourth, ninth, eleventh and twelfth nerves, respectively, in 1. These data indicating that the seventh nerve is most frequently involved correlate well with clinical observations.

The pathologic changes produced in the brain by leukemia may be briefly summarized by saying that the only changes in the brain parenchyma or the leptomeninges that are unques-

32. Tarro, E.: Pathologica **26**:609, 1934.

33. Fiessinger, N., and Marie, P. L.: Bull. et mém. Soc. méd. d. hôp. de Paris **27**:44, 1909.

34. Tapie, J., and Cassar, A.: Arch. d. mal. du cœur **12**:218, 1919.

tionably the result of the leukemic disease are hemorrhage and leukemic cell infiltration. All other changes described probably resulted from the infiltrations and the hemorrhages or from the agonal state. Only 5 of the cases (12, 14, 15, 17 and 20 in the table) did not show one or both of the two significant pathologic changes. Ninety-two per cent of all the cases included in the study and 83 per cent of the cases in the random series showed the two significant pathologic changes.

Location of the Lesions in the Brain.—Generally it was found that lesions were more frequent in the white matter, particularly the centrum ovale (fig. 1). In 41 of the 67 cases there were lesions in the cerebral hemisphere; in 21, in the medulla and pons; in 31, in the basal ganglions; in 19, in the cerebellum, and in 10, in the mesencephalon.

CLINICOPATHOLOGIC CORRELATIONS

It is noteworthy that with the high incidence of pathologic changes occurring in the brain in leukemia (92 per cent of all cases included in this study and 83 per cent of the cases in the random series), which in many instances included sizable leukemic infiltrations and extensive hemorrhages in the brain tissue and subarachnoid space, signs of increased intracranial pressure were but infrequently noted. Of the general signs of increased intracranial pressure, papilledema is the most reliable, since headache and vomiting may be symptoms caused by other manifestations of leukemia not directly related to any effect of this disease on the brain. Papilledema, noted in 5 of the cases (22, 23, 47, 51 and 65 in the table), was associated with leukemic infiltrations and hemorrhages in the brain of a sufficient degree to account for this finding in only 3 (cases 65, 51 and 47 in the table). In these 3 cases the immediate cause of death was believed to be the cerebral hemorrhage, and in addition significant leukemic infiltrations were found. In the remaining 2 cases (22 and 23 in the table) there were not sufficient lesions in the brain to account for papilledema. The case studied by Lengsfeld [35] (22 in the table) is a good example to illustrate the point that papilledema can occur without significant pathologic change in the brain. In this instance, besides the signs and symptoms of increased intracranial pressure, there were disturbances in equilibration, nystagmus, hyperactivity of the deep reflexes and loss of hearing, with only marked leukostasis, slight leukemic infiltrations in the leptomeninges and a hematoma of the right optic nerve sheath as pathologic changes in the brain to explain all of these neurologic signs and symptoms. Lengsfeld [35] postulated increased viscosity of the blood that caused anoxemia of the brain to explain the observed neurologic signs in his case. The adequacy or inadequacy of this explanation notwithstanding, this case does illustrate the point that in leukemia neurologic signs and symptoms are not always accompanied by demonstrable morphologic changes in the brain. Furthermore, it may be concluded from these observations that papilledema, although an infrequent sign in leukemia, cannot be taken, when present, as reliable evidence that there is concomitant significant pathologic change in the brain. The extremely low incidence of signs of increased intracranial pressure in the cases collected in this study, in which the incidence of pathologic changes in the brain is high, clearly indicates that lesions of the brain produced by leukemia but infrequently give signs and symptoms of a space-occupying lesion.

Anatomic lesions of the brain adequate to explain the hemiplegia observed in 11 of the cases (2, 4, 6, 15, 26, 27, 29, 33, 53, 54 and 57 in the table) were present in all except 1 (case 4 of the table). In this case, reported by Fränkel,[36] extensive hemorrhage and leukemic infiltration were described, but the description was not sufficiently detailed to locate a lesion that would adequately account for the hemiplegia. Of the 10 cases in which there was a localized lesion to account for the hemiplegia, it was found to be extensive destruction of the cerebral cortex in 5, a lesion of the internal capsule and the basal ganglions in 3 and an extensive lesion of the pons in 1.

It is noteworthy in view of the high incidence of pathologic changes occurring in the brain in leukemia that paralysis of cranial nerves was noted in only 12 cases and was statedly present in only 6 of the 11 cases in which hemiplegia was noted. It would seem fair to question the accuracy of the recording of involvement of cranial nerves in cases of leukemia since hemiplegic patients usually have involvement of cranial nerves more frequently than these figures would indicate. Furthermore, of the 27 cases in which a cerebral hemorrhage was the immediate cause of death, paralysis of cranial nerves was noted in only 6. The facial nerve was involved in all of the 12 cases in which paralysis of cranial nerves was noted, and in 5 instances,

35. Lengsfeld, W.: Jahrb. f. Kinderh. **126**:289, 1930.

36. Fränkel, A.: Ztschr. f. klin. Med. **3**:405, 1881.

was accompanied by paralysis of either the third, fifth, sixth, ninth or twelfth nerve. In only 3 cases, however, was there description of anatomic changes in the nerve or its nucleus. Kast [27] (case 7 in the table) described loss of nerve cells and degeneration of fibers in the facial nucleus in the case studied by him. In the case studied by Howell and Gough [29] (25 in the table), in which there was paralysis of both the facial and the oculomotor nerves, two small foci of leukemic infiltration were observed in the pons, as well as thrombosis of a blood vessel that ran through the facial nucleus. In addition, degeneration of fibers with infiltrations of leukemic cells was noted in the roots of the seventh and sixth cranial nerves. In the fifth case reported by Hellich [4] (28 in the table) a small focus of hemorrhage accompanied by leukemic infiltrations was observed in the medulla oblongata in the region of the facial nucleus to account for paralysis of the facial nerve.

It was not always possible to correlate anatomic changes occurring in the brain with alterations of reflexes. Changes in reflexes observed in 18 of the 64 cases in which there was record of reflexes were adequately explained by pathologic changes in the brain in only 10 instances. In cases studied by Schwab and Weiss [1] and that studied by Munro [37] lesions were present in the brain but the lesions were not in sufficient proportion to account for the changes in reflexes. There was no demonstrable pathologic change in the brain to account for alterations of reflexes in the case reported by Kast [27] (7 in the table), the case reported by Howell and Gough [29] (25 in the table), Hellich's [4] fifth case (28 in the table) and case 1 of our series (48 in the table). However, it is a well recognized fact in clinical neurology that changes in the reflexes may occur in systemic disease without demonstrable morphologic changes in the nervous system to explain them. Loss of the patellar reflex, for example, not infrequently accompanies lobar pneumonia and may occur together with a Babinski response in various types of coma.[38] It is reasonable that similar mechanisms to affect reflexes could be present in certain cases of leukemia to explain the presence of these clinical neurologic signs when demonstrable anatomic lesions are absent.

Convulsive manifestations observed in 6 of the cases (3, 19, 26, 28, 57 and 59 in the table) were in most instances accompanied by significant pathologic changes in the brain. Generalized convulsions were noted in 3 cases (3, 57 and 59 in the table), and there were extensive lesions in the brain to account for them in cases 3 and 57 but not in case 59. Unilateral convulsive types of involuntary movements were described by Hellich [4] in 2 of the cases reported by her (26 and 28 in the table), and in both there were significant lesions in the cortex and the thalamus. Rocchi [39] described unilateral clonic spasms of the facial muscles in the case he studied (19 in the table), in which hemorrhage and leukemic infiltrations were observed in the subcortical white matter beneath the convolutions in the parietal lobe of the contralateral side of the brain.

37. Munro, E. E. H.: J. A. M. A. **74**:603, 1920.

38. Purves-Stewart, J.: The Diagnosis of Nervous Diseases, Baltimore, William Wood & Company, 1937, p. 537.

COMMENT

Two points merit additional comment and consideration: first, the genesis and the incidence of the pathologic changes occurring in the brain in leukemia and, second, the incidence of clinical neurologic signs and symptoms caused by the leukemic disease.

It has been pointed out in the foregoing descriptions and discussion of the pathologic changes occurring in the brain in leukemia that the only pathologic changes that can be taken without question as absolutely characteristic and the direct result of the leukemic disease are hemorrhage and leukemic infiltration occurring independently or together in the brain, the dura mater or the leptomeninges. If hemorrhage and leukemic infiltration are taken as the criteria for pathologic change, it is found that these changes occurred in 26 of the 31 cases in the random series. Significant pathologic change was found in the brain in all of the other cases collected, but this is to be expected, since the reason for reporting these cases was that they presented unusual neurologic disease.

The leukemic infiltrations occurring in the brain in leukemia are undoubtedly similar to the infiltrations seen in other somatic tissues in this disease. It is generally believed that the cells in the foci of infiltration in the somatic tissues are principally derived from proliferating cells in the foci rather than that they have migrated there from the blood, since mitotic figures are seen in many of the cells. Mitotic figures were frequent in the foci of infiltration in the brains which we studied, and the character of the lesions strongly suggested that the locus of cells was expanding in a centrifugal direction, as there was usually a zone of necrotic tissue surrounding the infiltration (figs. 2 and 3). If all of the cells in the locus were derived from the blood, it would

39. Rocchi, F.: Policlinico (sez. med.) **33**:541, 1926.

seem unlikely that there would be such a tendency for the focus to be surrounded by necrotic brain.

The production of hemorrhages in the brain in leukemia is apparently the result of a combination of two factors. The first factor is the thrombopenia that usually accompanies leukemia and in itself is a good explanation of the hemorrhage occurring not only in the brain but in other somatic tissues in this disease. Our study suggests still another important factor in the genesis of the hemorrhages, and that is the part played by the foci of leukemic cells. It is highly significant that in 36 of the 47 cases in which there were foci of hemorrhage in the brain there was associated with the hemorrhage an infiltration of leukemic cells, indicating that the infiltration of cells in the brain is an important factor in the genesis of the hemorrhage, since hemorrhage occurred only infrequently without leukemic infiltration. Further support for this idea is obtained from the histologic study of the leukemic infiltrations, which showed in a high percentage of instances that the foci of infiltrates were surrounded by or diffusely infiltrated by red blood cells. It can be supposed therefore that foci of infiltration precede the hemorrhages, since in most instances the hemorrhage was of recent origin and it is unlikely that the amount of leukemic infiltration usually seen could have occurred following the hemorrhage. The mode of production of the hemorrhage by the leukemic infiltrations probably is that of a compression of the blood vessels within the foci from the proliferating leukemic cells causing necrosis and rupture of the wall. Bleeding once started in this way would be further augmented by the existing thrombopenia.

The incidence of neurologic signs and symptoms in cases of leukemia and their incidence in the cases of leukemia in which pathologic change has occurred in the brain are important questions that can be answered from this study. Our study indicates that there were symptoms of neurologic disease in 11 of the 31 cases in the random series. Although the number of cases is not large, this incidence of 35 per cent may be taken as a fair index of the occurrence of neurologic disease in leukemia. When compared with the 83 per cent incidence of pathologic changes in the brain in this same series of cases it emphasizes the fact that an appreciable number of patients with leukemia having significant pathologic changes in the brain never manifest neurologic signs or symptoms. The incidence of such signs and symptoms in our series is higher than that reported by Schwab and Weiss,[1] who observed that they appeared sometime during the course of the disease in 20 per cent of 334 cases of leukemia. The greater number of cases in the study of Schwab and Weiss would give greater reliability to their observation, although it is not known in how many of the cases pathologic changes were present in the brain, since in only 3.3 per cent of the instances was there pathologic study of this organ.

Of the 62 cases in which significant pathologic change was observed in the brain, 33 were marked by neurologic signs or symptoms. In the random series, 8 of the 26 cases with pathologic change in the brain were marked by signs and symptoms of neurologic disease. The discrepancy between the incidence of neurologic disease in the two groups of cases can probably be explained by the fact that in most of the cases the reason for the recording was that unusual neurologic features were present. Moreover, it has been previously pointed out from this study that significant pathologic changes may be present in the brain in leukemia without neurologic signs or symptoms. It may be safely concluded from these figures that approximately 30 per cent of patients with leukemia who have significant pathologic change in the brain will manifest neurologic signs and symptoms.

SUMMARY

A clinical and pathologic study of 20 brains selected at random from persons dead of leukemia has been presented to determine the type and the extent of the pathologic changes occurring in the brain in this disease and to correlate them with the clinical neurologic signs. The gross pathologic study of the brain was carried on as the organ was serially sectioned by means of a slicing machine, the individual slices being not more than from 3 to 5 mm. thick.

In a review of the literature, 47 cases of leukemia having adequate pathologic study of the brain have been collected; to this group we have added the 20 cases on which the aforementioned study was based. Of the 67 cases collected for this study, only 31 could be considered as selected at random. The clinical and pathologic features of leukemia as it affects the brain have been determined from an analysis of these cases.

Hemorrhage and leukemic cell infiltration in the brain, occurring separately or together, were the only significant pathologic changes that could be directly attributed to the leukemic disease. One or both of these pathologic changes were present in 62 of the cases. They were observed in 26 of the 31 cases that could be regarded

as selected at random. Grossly visible foci of hemorrhage were present in 46 of all the cases and in 19 of the cases in the random series. Infiltrations of leukemic cells in the brain were described in 42 cases, and in 36 of these cases there was hemorrhage in or surrounding the foci of infiltration. Hemorrhage of a proportion to be the immediate cause for death was present in the brain in 27 of all of the cases and in 9 of the 31 cases in the random series.

The foci of leukemic cells occurring in the brain in leukemia are similar in all respects to those seen in other somatic tissues in this disease and represent foci of proliferating leukemic cells in the tissue. The infiltrations of leukemic cells in the brain are thought to be important factors in the production of the hemorrhages in the brain in leukemia, since hemorrhage without an accompanying infiltration with leukemic cells was infrequent. Both hemorrhage and leukemic infiltration in the brain show a greater predilection for the white matter than for the gray matter. There does not appear to be any significant difference in the production of lesions in the brain and the development of neurologic signs and symptoms between the different types of leukemia, although hemorrhage and leukemic infiltration were slightly more frequent in the chronic than in the acute forms of leukemia.

Neurologic signs and symptoms attributable to the leukemic disease were present in 34 of all of the cases included for study but appeared in only 11 of the 31 cases in the random series. Neurologic symptoms were the first clinical manifestation of the leukemic disease in 5 of the cases. Hemiplegia was observed in 11 of the cases and was always accompanied by a hemorrhage in the brain of a proportion to be the immediate cause of death. In rare instances neurologic signs and symptoms were exhibited without demonstrable pathologic change in the brain. Signs and symptoms of increased intracranial pressure sufficient to indicate a space-occupying intracranial lesion were only rarely observed, in spite of the fact that in many instances the infiltrations of leukemic cells involved large parts of the brain.

CONCLUSIONS

Approximately 80 per cent of patients with leukemia have significant pathologic changes in the brain consisting of hemorrhages and infiltrations of leukemic cells. Neurologic signs and symptoms are present in from 20 to 35 per cent of patients with this disease. Hemorrhage into the brain in leukemia sufficiently extensive to be the immediate cause for death occurs in approximately 29 per cent of the patients.

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COMPARATIVE EFFICACY OF PANCREATIN AND PEPSIN IN THE EXPERIMENTAL PRODUCTION OF INTESTINAL ULCERS

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In previous communications my associates and I [1] have reported the results of studies of the effectiveness of various chemicals, of hydrostatic pressure and of the concentration of pepsin in the experimental production of peptic ulcers in the intestines of dogs. The role of pepsin in the causation of ulcers in the gastrointestinal tract has been extensively investigated, but little attention has been given to the possibility that other enzymes have an effect in the production of the lesions. That such a possibility exists is suggested by the fact that bile and the secretions of the intestine and the pancreas quickly decrease the acidity of gastric juice which is necessary for peptic activity.

The purpose of the experiments reported here was to determine what conditions, if any, lead to the production of ulcers by pancreatin and to compare the potency of this enzyme with that of pepsin in producing necrosis in the intestines.

PROCEDURE

The abdomens of 107 anesthetized dogs [2] were opened, and in each dog a loop of intestine about 18 inches (45.5 cm.) long, reaching from the midportion of the duodenum to a point in the jejunum 12 inches (30.5 cm.) below the muscle of Treitz, was cannulated at each end for the introduction and removal of solutions to be tested. The loop was reinserted into the abdomen and exposed to the solution, which was renewed every hour. The time of exposure ranged from four to twenty hours or until perforation occurred. The loop was then removed, split along the mesenteric line and examined grossly and microscopically. The numbers 1 to 4 were used in recording the severity of the damage as follows: 1 was used to represent a condition in which there is slight surface necrosis of the mucosa; 2, to designate deeper necrosis involving at least one third of the thickness of the mucosa over most of the area, with smaller areas (from 0.5 sq. cm. to 2 sq. cm.) showing necrosis as deep as the submucosa; 3, to indicate necrosis of practically all the mucosa, with some patches extending into the circular muscle, and 4, to represent a loop having areas of necrosis extending to the serous coat or to a point of frank perforation. In each group of experiments the average of the scores was taken.

RESULTS

The solutions to which the loops were exposed are given in the first column of the accompanying table and the damage produced by them in columns 5 and 8. The periods of time required to produce this damage are found in columns 4 and 7.

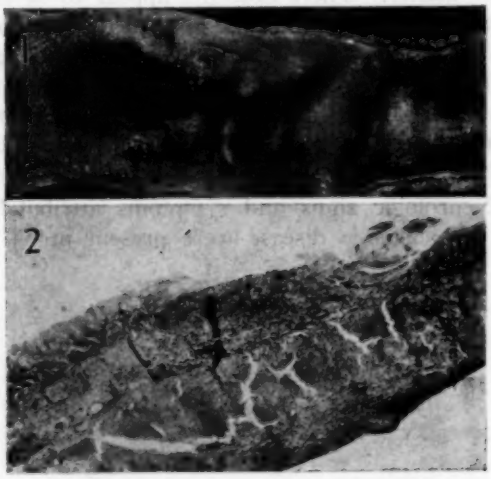

Fig. 1.—Segment of jejunum with multiple ulcers of the mucosa. The dark region represents an area of hemorrhage.

Fig. 2.—Photomicrograph of a section through the margin of an ulcer; × 24. The ulceration extends into the submucosa at one point almost to the muscularis.

Both enzymes, pancreatin and pepsin, were tried in alkaline and in acid solution at hydrogen ion concentrations corresponding to those of the secretions of the pancreas and the stomach, respectively.

In a recent paper [1e] from this laboratory it was demonstrated that hydrostatic pressure markedly increases the extent of peptic digestion in the

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1. (*a*) Driver, R. L.; Dozier, G. S., and Denham, H. C.: Science **98**:158, 1943. (*b*) Driver, R. L.; Chappell, R. H., and Carmichael, E. B.: Am. J. Digest. Dis. **12**:166-167 (May) 1945. (*c*) Driver, R. L.; Chappell, R. H., and Carmichael, E. B.: ibid. **12**:168-169 (May) 1945. (*d*) Chappell, R. H.; Driver, R. L., and Carmichael, E. B.: ibid. **12**:169-171 (May) 1945.

2. The anesthetic used was Solution Dial with Urethane, supplied by the Ciba Pharmaceutical Products, Inc., Summit, N. J.

production of ulcers, and since there is considerable variability in the intraintestinal pressure of dogs and man [3] (the pressure sometimes exceeding 150 cm. of water [3e]), about half the loops were exposed under a pressure of 90 cm. of water.

Degrees of Damage of Intestinal Loops Exposed to Various Solutions in the Absence of Pressure and at a Pressure of 90 Cm. of Water

Solution	p_H	In Absence of Presure: Dogs	In Absence of Presure: Time Exposed, Min.	In Absence of Presure: Severity of Damage *	At a Pressure of 90 Cm. of Water: Dogs	At a Pressure of 90 Cm. of Water: Time Exposed, Min.	At a Pressure of 90 Cm. of Water: Severity of Damage *
N/10 $NaHCO_3$	8.1	3	630	0	6	642	0
0.1% pancreatin † in N/10 $NaHCO_3$	8.1	6	780	0	12	623	1.8
0.1% pepsin ‡ in N/10 $NaHCO_3$	8.1	3	600	0	5	650	0
N/10 HCl	1.0	17	700	0.5	12	174	4.0
0.1% pancreatin † in N/10 HCl	1.2	6	750	0.5	12	185	4.0
0.1% pepsin ‡ in N/10 HCl	1.2	15	700	1.9	10	82	4.0
(1)		(2)	(3)	(4)	(5)	(6)	(7)	(8)

* Each value represents an average of the scores on damaged loops with severity being graded 1 to 4.
† The pancreatin used was from Pfanstiehl.
‡ The pepsin used was from Merck & Company, Inc.

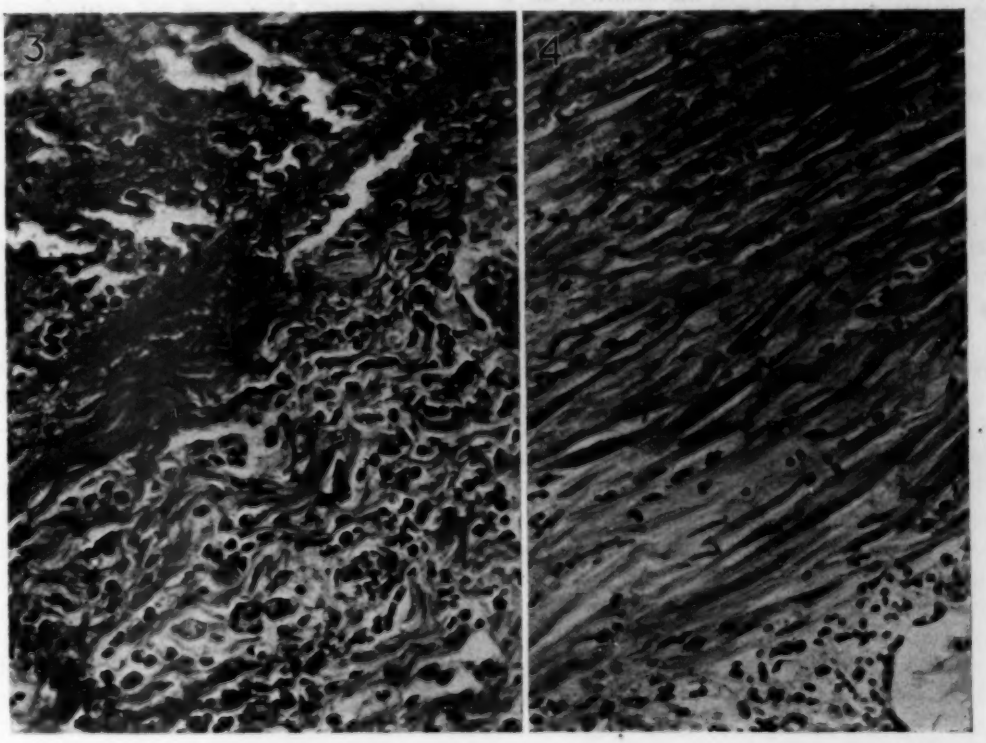

Fig. 3.—Photomicrograph showing the mucosa and the submucosa at the margin of the ulcer; × 332. The blood vessels are distended with erythrocytes, and the tissue is infiltrated with polymorphonuclear neutrophilic leukocytes.

Fig. 4.—Photomicrograph through the adjoining muscularis showing the infiltration of polymorphonuclear neutrophilic leukocytes; × 332.

COMMENT

In tenth-normal sodium bicarbonate neither pancreatin nor pepsin caused any damage in the absence of pressure, but with these enzymes in tenth-normal hydrochloric acid damage was produced as seen in the table. However, in the case of pancreatin the damage was not due to

3. (*a*) Abbott, W. O.; Hartline, H. K.; Hervey, J. P.; Ingelfinger, F. J.; Rawson, A. J., and Zetzel, L.: Am. J. M. Sc. **199**:879, 1940. (*b*) Sherrington, C. S.: Brain **38**:191, 1915. (*c*) Owing, J. C.; McIntosh, C. A.; Stone, H. B., and Weinberg, J. A.: Arch. Surg. **17**:507, 1928. (*d*) Sperling, L.; Paine, J. R., and Wangensteen, O. H.: Proc. Soc. Exper. Biol. & Med. **32**:1504, 1935. (*e*) Stone, H. B., and Firor, W. M.: Tr. South. S. A. **37**:173, 1924.

the enzyme but to the acid, as shown by control experiments in which acid alone was used. This observation is a confirmation in vivo of experiments in vitro showing that the optimal medium for pancreatin activity is an alkaline one.[4]

When the pressure was 90 cm. of water, neither tenth-normal sodium bicarbonate alone nor pepsin in tenth-normal sodium bicarbonate caused any damage, but pancreatin in tenth-normal sodium bicarbonate did have an effect (figs. 1, 2, 3 and 4). Of 12 intestinal loops in which this solution was used, 2 showed no damage, 1 was damaged only to the extent of having the simple columnar epithelial covering of three Peyer's patches digested off and the remaining 9 showed necrosis reaching all degrees of damage up to the presence of more than 100 ulcers from 0.1 cm. to 1 cm. in diameter (in 1 loop). However, there were no perforated ulcers in these loops. Acid solutions under a pressure of 90 cm. of water had the most pronounced effect of all. Tenth-normal hydrochloric acid brought about perforation in one hundred and seventy-four minutes, pancreatin in tenth-normal hydrochloric acid in one hundred and eighty-five minutes and pepsin in this solution in eighty-two minutes.

4. Harrow, B.: Textbook of Biochemistry, ed. 4, Philadelphia, W. B. Saunders Company, 1944, p. 118.

SUMMARY

Pancreatin in tenth-normal sodium bicarbonate produces ulcers in the intestines of dogs under a hydrostatic pressure of 90 cm. of water in ten hours. It has no effect in the absence of intraintestinal pressure, and it is without effect in tenth-normal hydrochloric acid with or without pressure.

Pepsin in tenth-normal sodium bicarbonate produces no ulcers with or without pressure.

EXPERIMENTAL NEPHROPATHIES

III. CALCIFICATION AND PHOSPHATASE IN THE KIDNEYS OF DOGS POISONED WITH MERCURY BICHLORIDE, POTASSIUM DICHROMATE AND URANYL NITRATE

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Robison [1] found large amounts of phosphatase in cartilage that was in the process of ossification and was the first to suggest that it is concerned in the calcification of bone. Later Martland and Robison [2] added further evidence in support of this suggestion. More recently Gomori [3] stated that "calcification of living or recently necrosed tissue seems to start invariably in the centers of intensely phosphatase-positive areas." However, his study of the calcification of necrotic tissue appears to have been limited to foci of tuberculosis. Since calcification occurs frequently in the kidneys and these organs are rich in phosphatase, it is remarkable that there is so little in the literature concerning the possible relation between renal calcification and the presence of the enzyme in the epithelium of the renal tubules. Harris [4] many years ago stated that renal phosphatase was responsible for calcification in the kidneys. This statement was promptly questioned by Kay.[5] Wilmer [6] recently declared that the phosphatase of the kidneys is in no way related to calcification in these organs. Our studies have a definite bearing on this disputed point but have also a much wider significance.

Calcium is deposited in the kidneys of dogs poisoned with mercury bichloride, uranyl nitrate or potassium dichromate only when the dose is sufficiently large to cause extensive necrosis of the tubular epithelium. Among the dogs used in our experiments,[7] 28 had sufficient necrosis of the renal tubules to lead one to expect calcification. In the kidneys of these dogs with approximately equivalent degrees of necrosis, the extent of the accompanying calcification varied with the chemical agent used. The quantity of calcium in the renal cortex was determined chemically, and sections of kidneys were stained with hematoxylin and eosin, by von Kossa's method and, in some instances, with alizarin. Phosphatase was revealed in the tissues qualitatively by Gomori's [8] staining method and quantitatively by Bodansky's [9] chemical method. The results are shown in table 1.

Mercury bichloride administered to dogs causes quantitative changes in the calcium, the phosphatase and the phosphorus of the blood. In one group of dogs the blood levels of these substances were determined before, and two or more times after, poisoning with the bichloride. In table 2 the means of the maximum and minimum values of these three factors are compared with the mean of the control values obtained before administration of the poison.

The means of the maximum values after poisoning were considerably higher than the means of normal control values obtained before administration of the poison. The mean maximum value of calcium was 19.75 per cent, of phosphatase 39.40 per cent, and of phosphorus 22.45 per cent, respectively, above the corresponding normal means. The mean of the minimum values of phosphatase was 27.40 per cent below the normal mean, while the means of the minimum values of calcium and phosphorus were within the limits of probable error of the normal mean for each. This increase of the calcium of the blood after poisoning with mercury bichloride may be a factor in the marked calcification of the kidneys after poisoning with this substance. Some of the excess calcium of the blood plasma must have been excreted by the kidneys, for the glomeruli of the kidneys of these dogs appeared quite capable of functioning.

The amount of calcium in the kidneys of dogs poisoned with mercury bichloride varied with

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1. Robison, R.: Biochem. J. **17**:286, 1923.
2. Martland, M., and Robison, R.: Biochem. J. **21**: 665, 1927.
3. Gomori, G.: Am. J. Path. **19**:197, 1943.
4. Harris, L. J.: Lancet **1**:1031, 1932.
5. Kay, H. D.: Lancet **1**:1383, 1932.
6. Wilmer, H. A.: Arch. Path. **37**:227, 1944.
7. Simonds, J. P., and Hepler, O. E.: Arch. Path. **39**:103, 1945.
8. Gomori, G.: Proc. Soc. Exper. Biol. & Med. **42**:23, 1939; J. Cell. & Comp. Physiol. **17**:71, 1941.
9. Bodansky, A.: J. Biol. Chem. **101**:93, 1933.

the dose administered and roughly with the time that elapsed between its administration and the death of the animal. The results with mercury bichloride are shown in table 3. In dogs receiving 3 mg. of mercury bichloride per hundred cubic centimeters of blood the mean value of renal phosphatase was 15.11 per cent below, while in those given 2 mg. or less it was 54.57 per cent above, the normal mean. In 3 dogs receiving 3 mg. of mercury bichloride the average value of the calcium of the kidneys was 30 per cent above, and in 4 dogs given 2 mg. or less it was 13.95 per cent below, the normal mean. In dogs given 2 mg. of mercury bichloride the mean value of renal phosphorus was 51.78 per cent above, and in those receiving 2 mg. or less it was 30.36 per cent above, the normal mean.

TABLE 1.—*Interrelations of Necrosis, Calcium and Phosphatase in the Kidneys of Dogs Damaged by Chemical Agents*

Dog	Dose, Mg.	Necrosis	Calcium	Phosphatase: Stain	Phosphatase: Chemical Units	Urine in Bladder at Death: Amount	Urine in Bladder at Death: Albumin
Hg* 3	3 × 1	++	+	+++	6.12	+	+++
Hg 5	1 × 3	++++	+++	+++	4.28	+	++++
Hg 6	1 × 3	++++	+++	++++	11.06	+	++++
Hg 7	1 × 3	++++	++++	+++	8.47	+	++++
Hg 8	1 × 3	++++	++++	++++	11.31	0	
Hg 9	1 × 3	++++	++++	.....	4.99	+	+++
Hg 10	1 × 3	++++	++++	+++	5.00	+	+++
Hg 12	1 × 3	++++	++	.....	....	0	
Ur* 1	2 × 1	+++	0	.....	....	++	+++
Ur 2	3 × 1	++++	+	.....	....	++	++++
Ur 3	3 × 1	++++	0	.....	....	++	+++
Ur 8	2 × 1	++	0	++++	18.35	++	+++
Ur 10	1 × 2	++	0	++++	15.04	+	+++
Ur 11	1 × 2	+++	0	++++	15.21	+	+++
Ur 12	1 × 2	++++	+	+++	14.77	++	+++
Ur 13	1 × 2	+++	0	+++	18.09	+	+++
Ur 20	2 × 3	++++	+++	++++	14.44	++	++++
Ur 21	2 × 3	++++	++	.....	14.07	+	++++
Ur 22	2 × 3	++++	++	++++	.....	++	++++
Cr* 1	3 × 1	++	0	.....	....	++	++++
Cr 3	3 × 1	+++	0	.....	....	++	+++
Cr 8	2 × 2	++++	0	+++	6.00	+	++++
Cr 13	1 × 2	+++	0	+++			
Cr 16	2 × 3	+++	++	++++	6.65	++	++++
Cr 17	2 × 3	++++	++	+++	7.40	++	++++
Cr 18	2 × 3	++++	0	+++	9.60	++	++++
Cr 19	2 × 1	++	0				
Cr 20	2 × 1	++	0				

* Hg dogs were poisoned with mercury bichloride ($HgCl_2$).
Ur dogs were poisoned with uranyl nitrate ($UrO_2[NO_3]_2$).
Cr dogs were poisoned with potassium dichromate ($K_2Cr_2O_7$).

TABLE 2.—*Changes in the Mean Values of the Calcium, the Phosphatase and the Phosphorus of the Blood Before and After Poisoning with Mercury Bichloride*

	Dogs	Mean of Control Values Before Poisoning	Mean of Maximum Values After Poisoning	Mean of Minimum Values After Poisoning
Calcium, mg. per 100 cc.	12	9.92 ± 0.21	11.87 ± 0.30	0.96 ± 0.16
Phosphatase, Bodansky units per gram	10	3.50 ± 0.31	4.88 ± 0.37	2.54 ± 0.11
Phosphorus, mg. per 100 cc.	10	4.90 ± 0.23	6.00 ± 0.31	4.63 ± 0.32

Of 8 dogs poisoned with mercury bichloride (table 1), 6 died in from one to six days. The other 2 (Hg 3 and Hg 8) were killed after four and seven days, respectively. Extensive necrosis and moderate to marked calcification were present in all 8 dogs (100 per cent) of this group. In the animal with the smallest amount of necrosis (Hg 3) the calcium was deposited as fine granules in isolated cells, the adjacent cells in the necrotic tubule being free. A dog that survived only twenty-four hours (Hg 5) had marked calcification in the kidneys (figs. 1, 2 and 3). In the dog in which the calcification was most marked (K Hg 8) the calcium was deposited in coarse, blocklike masses and sometimes appeared almost crystalline (figs. 4, 5 and 6). Two of the dogs (Hg 9 and Hg 10) were in the group for which table 1 was made, and they had the highest maximum blood calcium values in that group, namely, 32.2 per cent and 51.7 per cent, respectively, above the normal mean for the group.

TABLE 3.—*Comparison of Mean Values of Renal Calcium, Phosphatase and Phosphorus in Control Dogs and in Dogs Poisoned with Varying Doses of Mercury Bichloride*

	Dogs	Mean Values for Normal Controls	Dogs	Mean Values with 3 Mg. $HgCl_2$	Dogs	Mean Values with 2 Mg. $HgCl_2$ or Less
Calcium, mg. per 100 cc.	16	10.75 ± 0.56	3	13.98	4	9.25
Phosphatase, Bodansky units per gram	37	10.72 ± 0.33	10	9.10 ± 0.75	7	16.57 ± 1.39
Phosphorus, mg. per 100 cc.	37	1.12 ± 0.02	10	1.70 ± 0.11	7	1.46 ± 0.08

Renal phosphatase was determined chemically for 7 of the aforementioned 8 dogs (table 1). Of these, 5 had a low phosphatase content, averaging 5.89 Bodansky units, which is 45 per cent below the normal mean. In the other 2 dogs the renal phosphatase averaged 11.19 units, which is within the limits of probable error of the normal mean. Sections of the kidneys of 6 of the dogs in this group were stained by Gomori's method. All had an abundance of phosphatase in the labyrinth, that is, in the upper segments of the proximal convoluted tubules, where it was present in its usual normal location in the brush border of the cells. There was much less demonstrable enzyme in the lower segments of these tubules (figs. 2 and 5).

In 6 of these dogs the urine found in the bladder was scanty and contained much albumin.

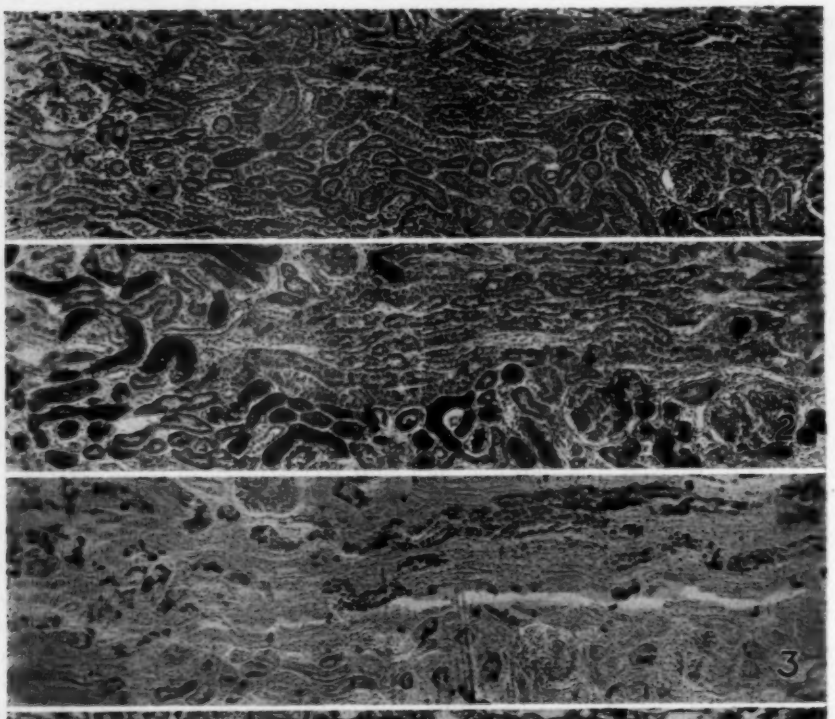
In 2 the bladder was empty. Since the glomeruli of the kidneys of the dogs in this group appeared structurally normal, there is no reason to assume that the oliguria and anuria of these animals were the result of failure of glomerular filtration. It is more likely that the filtrate diffused through the mass of necrotic cells into the peritubular capillaries, leaving much of its calcium deposited in the intratubular necrotic material.

Nine dogs poisoned with potassium dichromate showed extensive necrosis of the tubular epithelium of the kidneys and none died during the period of the experiments. Only 2 of these dogs (22.2 per cent) had deposits of calcium in the necrotic tubules, and this only in finely granular form. The kidneys of these 2 dogs contained an average of 7.03 units of phosphatase per gram of tissue (34.4 per cent below the normal mean). Sections of the kidneys of all these dogs stained by Gomori's method showed the enzyme in the necrotic material in the upper segments of the proximal convoluted tubules (fig. 8). In figure 8 the phosphatase is diffused uniformly through the necrotic material in some of the tubules, while in other necrotic tubules there is no enzyme. Since figures 7, 8 and 9 are not from serial sections but only from the same block of tissue, it is not possible to determine whether the calcium in figure 9 is in necrotic material that still contains active phosphatase. It may be significant, however, that the proportion of tubules with to those without calcification in figure 9 is approximately equal to the proportion of tubules without to those with phophatase in figure 8. The quantities of urine found in the bladders of 6 of these dogs were recorded. The urine was more abundant than that in the bladders of the mercury bichloride group and contained much albumin.

Eleven dogs poisoned with uranyl nitrate showed extensive necrosis of the tubular epithelium, but none of these died within the duration of the experiments. Of these, only 5, or 45 per cent, showed demonstrable calcification of the kidneys. In all these calcium was present in the form of fine granules within the necrotic cells. In 2 the deposits of calcium were scanty and limited to single isolated cells. In 3 of these 5 dogs the kidneys contained an average of 14.43 units of phosphatase (34.6 per cent above the normal mean). Sections of the kidneys of 7 dogs of this group were stained by Gomori's method, and all contained much of the enzyme in the labyrinth but practically none in the lower straight segments of the proximal convoluted tubules (fig. 11). In the kidneys of dogs poisoned with uranyl nitrate the calcium was deposited in the straight portions of the tubules and not in the labyrinth (fig. 12). The quantities of urine found in the bladders of 6 of these dogs were recorded. The urine was more abundant than in the bladders of the mercury bichloride group and contained much albumin.

Mercury bichloride, uranyl nitrate and potassium dichromate in sufficient doses produce extensive necrosis of the epithelium of the proximal convoluted tubules. When the necrosis is of marked extent, calcification may be present in kidneys of dogs poisoned with any one of these substances. But necrosis alone is not the chief factor, nor is the presence of active phosphatase, for among dogs with approximately equal amounts of necrosis the kidneys of 22.2 per cent of those poisoned with potassium dichromate, 45 per cent of those poisoned with uranyl nitrate and 100 per cent of those poisoned with mercury bichloride showed calcification. The kidneys of all these animals, in spite of the necrosis, still contained active phosphatase, demonstrable by chemical and microtechnical methods (figs. 2, 5, 8 and 11). In poisoning with potassium dichromate the enzyme was in the necrotic material that filled the lumen, and it was in this material that the calcium was deposited. In other words, the calcium and the phosphatase were both present in the same general location, i. e., in the labyrinth but, as pointed out in a foregoing paragraph, not necessarily in the same necrotic tubules (figs. 8 and 9). However, in the kidneys of dogs poisoned with uranyl nitrate and with mercury bichloride the calcium was deposited chiefly in the terminal portions of the proximal convoluted tubules, and in these segments the amount of phosphatase, as demonstrated by Gomori's method, was much reduced and completely inactivated (figs. 2, 3, 5 and 6). In the dogs poisoned with mercury bichloride and potassium dichromate the quantity of calcium and the activity of phosphatase in the kidneys, determined chemically, varied inversely.

As pointed out in another paper in this series [7] the terminal portions of the proximal convoluted tubules contain fat in relatively large amount in a high percentage of normal dogs. The percentage of animals with stainable renal fat was much greater in the groups poisoned with these three substances than in the control group of normal animals or in those given injections of what were called in the previous paper glomerular poisons. It is likely that fatty degeneration of the tubular epithelium of varying degrees occurs in all kidneys damaged by these inorganic poisons. The presence of fat may therefore be a factor in the



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calcification of these organs under the conditions of these experiments.

The fact that mercury bichloride and uranyl nitrate produce their primary necrotizing effects in the lower segments of the proximal convoluted tubules, where fat is most abundant, while potassium dichromate damages the upper parts of these tubules, may be a significant factor in the more frequent occurrence and the greater extent of calcification in the kidneys of dogs poisoned with the first two of these three substances. Furthermore, in some of these dogs (e. g., Hg 10 and Ur 20 and 21) the dose of the poison was so large that fatty changes were found in the glomeruli. In sections of the kidneys of these dogs stained by von Kossa's method, fine dustlike particles, probably calcium, were present in the glomeruli.

In sections stained with sudan and counterstained with hematoxylin and eosin, red (fat) and dark blue (calcium) droplets were frequently seen in the same cells. Furthermore, in some sections many red droplets of fat appeared to have a pale bluish tinge as if there was a fine deposit of calcium on their external surfaces. Klotz [10] observed a similar coating of fat droplets with calcium in the tubular epithelium of rabbits poisoned with heavy metals. Hartsuch [11] found that calcium soaps are formed by an interchange of ions at the water-oil interphases when immiscible fats and basic substances are present in aqueous systems. Insoluble calcium salts resulted from further molecular changes. But even this does not fully account for the marked differences in the extent of calcification in dogs poisoned with the three substances used in our experiments. The mechanisms of this difference must be sought in other factors.

The most likely source of the calcium deposited in the kidneys of these dogs is the glomerular filtrate. In comparison with potassium dichromate and uranyl nitrate, mercury bichloride causes more marked oliguria, even anuria, and less calcium escapes in the urine. It is possible, therefore, that in poisoning with mercury bichloride the glomerular filtrate diffuses into the peritubular tissue spaces and thence into the peritubular capillaries to a much greater extent than occurs in poisoning with potassium dichromate and uranyl nitrate. In mercury bichloride poisoning, also, the calcium of the blood and presumably that of the glomerular filtrate are increased. In dogs Hg 9, 10 and 12 the maximum blood calcium found in multiple determinations after poisoning with mercury bichloride was 13.11, 15.05 and 13.30 mg. per hundred cubic centimeters of blood, respectively. These values average 13.82 mg., which is 39.21 per cent above the mean blood calcium value of the normal controls. With calcium diffusing through the necrotic material, particularly in the presence of fat, conditions would be more favorable for its deposition in the kidneys of dogs poisoned with mercury bichloride than in those poisoned with the other two substances. This appears to be a reasonable explanation of the greater amount of calcification in the kidneys of dogs poisoned with mercury bichloride. As long ago as 1890 Chittenden and Lambert,[12] and much later others, such as Milhorat and Deuel [13] and Hayman and co-workers,[14] reported initial polyuria lasting two or three days, followed by oliguria, after poisoning with salts of uranium. A period of polyuria also occurs after poisoning with mercury bichloride but more quickly gives place to oliguria or anuria. This initial increase in the output of urine is probably a factor in the smaller amount of calcification in the kidneys of dogs poisoned with necrotizing

10. Klotz, O.: J. Exper. Med. **7**:633, 1905.
11. Hartsuch, P. J.: Arch. Path. **25**:17, 1938.
12. Chittenden, R. H., and Lambert, A.: Tr. Connecticut Acad. Arts & Sc. **7**:261, 1890.
13. Milhorat, A. T., and Deuel, H. J.: Arch. Int. Med. **60**:77, 1937.
14. Hayman, J. M.; Shumway, N. P.; Dumke, P., and Miller, M. D.: J. Clin. Investigation **18**:195, 1939.

EXPLANATIONS OF FIGURES 1 TO 6

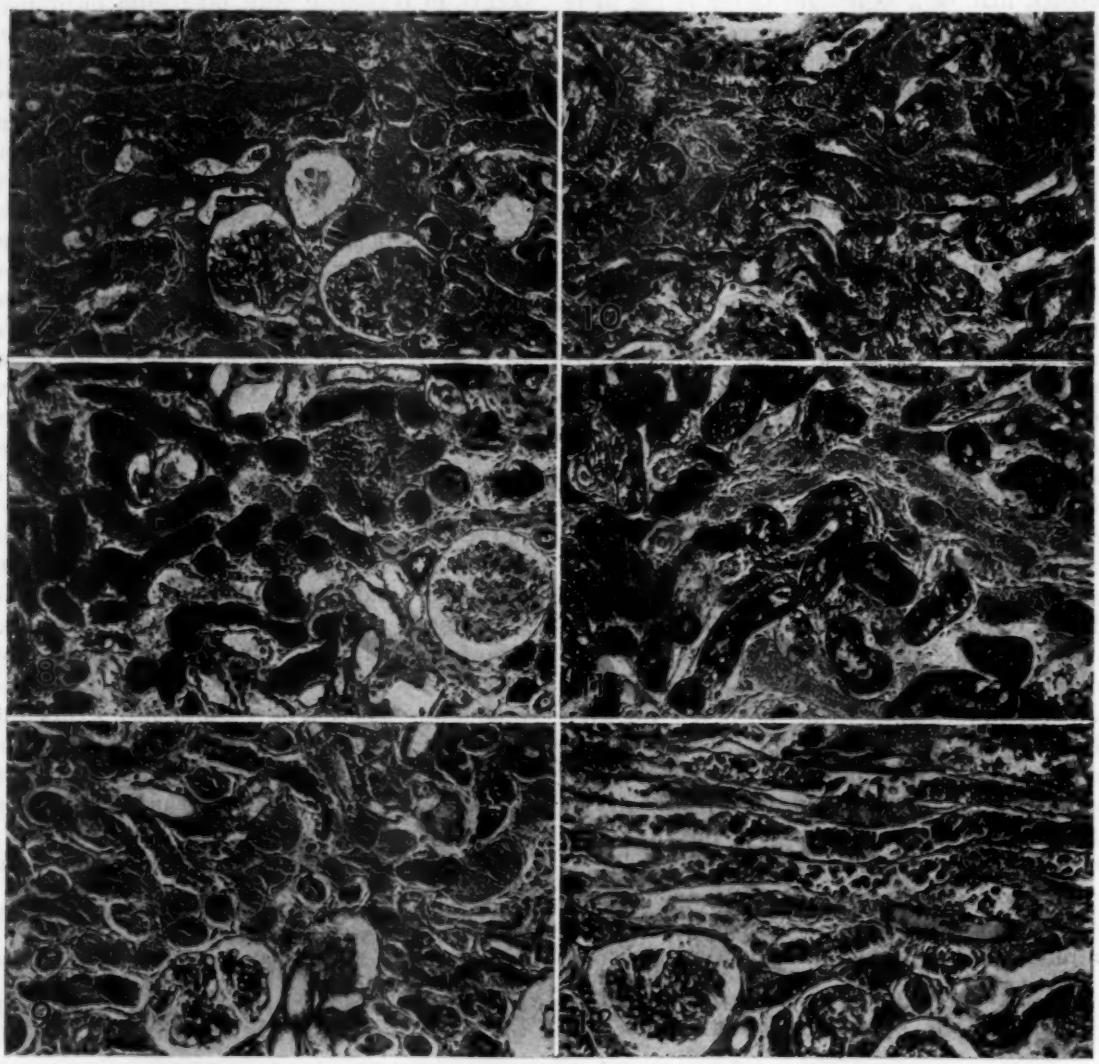
Figs. 1, 2 and 3.—Serial sections (× 55) of a kidney from dog K Hg 5, which received one dose of 3 mg. of mercury bichloride ($HgCl_2$) per hundred cubic centimeters of blood and died in twenty-four hours. In section 1, stained with hematoxylin and eosin, necrosis and calcification are seen in the straight portions of the tubules. Section 2 was stained by Gomori's method and shows phosphatase in the tubules in the labyrinth and a very slight amount in the straight portions of the tubules. In section 3, stained by von Kossa's method, the calcium is deposited in individual cells in the straight portions of the tubules. The active phosphatase and the calcium are definitely in different parts of the sections. In sections of the same kidney stained with sudan, the calcium-containing portion of the tubules contained fat.

Figs. 4, 5 and 6.—Serial sections (× 55) from the kidney of dog K Hg 8, which received one injection of 3 mg. of mercury bichloride per hundred cubic centimeters of blood and was killed on the seventh day. Section 4 was stained with hematoxylin and eosin, section 5, by Gomori's method and section 6 by von Kossa's method. Phosphatase is most abundant in the tubules on either side of, above and below the small artery in the right upper quadrant in figure 5. Calcium is limited almost entirely to the left half of the section in figure 6 and is present in coarse masses in necrotic material. In other sections of this kidney stained with sudan, fat was present in the necrotic material containing calcium.

doses of uranyl nitrate, since more calcium is lost in the urine.

It is probable, also, that some of the phosphatase found in the necrotic material in the tubules of the 28 dogs in this series may have come from the glomerular filtrate. This appears to be particularly true in dogs poisoned with potassium dichromate, in which the necrotic material in the tubules was rich in phosphatase (fig. 8).

Our studies do not support the view that phosphatase is concerned with calcification in the kidneys of the dog. In the first place, phosphatase acts only on the esters of phosphoric acid and



Figs. 7, 8 and 9.—Sections (× 155) of a kidney from dog K Cr 16, which received two injections of 3 mg. each of potassium dichromate ($K_2Cr_2O_7$) per hundred cubic centimeters of blood and was killed on the seventh day. In section 7, stained with hematoxylin and eosin, there is complete necrosis in most of the tubules in the labyrinth. In section 8, stained by Gomori's method, phosphatase is abundant and is diffused uniformly through the necrotic material in most of the tubules. In the right lower quadrant is a small group of tubules with necrosis but little or no phosphatase. In section 9 calcium is seen irregularly deposited in some of the tubules. The number of necrotic tubules with calcium in this section is roughly proportional to the number without phosphatase in section 8. This suggests that the calcium is deposited in necrotic cells with little or no active phosphatase.

Figs. 10, 11 and 12.—Sections (× 155) from a kidney of dog K Ur 21, which received two injections of 3 mg. each of uranyl nitrate ($UrO_2[NO_3]_2$) per hundred cubic centimeters of blood and was killed on the seventh day. In section 10, stained with hematoxylin and eosin, the epithelium of the straight portions of the tubules is necrotic and that in the labyrinth is markedly swollen. In section 11, stained by Gomori's method, the necrotic epithelium contains no phosphatase, while this enzyme is diffusely distributed in the cytoplasm of the cells in the labyrinth. In section 12, stained by von Kossa's method, calcium is present in the straight portion of the tubules with little or none in the labyrinth. There is thus a sharp difference in the distribution of calcium and phosphatase in this kidney.

not on inorganic phosphates. Only about 6 per cent of the phosphates of the urine are of organic origin, i. e., from glycerophosphates, hexophosphates and neucleotides.[15] Second, in our experiments the quantity of calcium and the phosphatase activity in the kidneys of dogs poisoned with mercury bichloride and with potassium dichromate varied inversely. In most of the animals in which the phosphatase activity of the kidneys, determined by chemical means, was increased, the amount of calcium deposited was minimal. Third, with roughly comparable amounts of necrosis and of stainable phosphatase, calcium was deposited in the kidneys of 100 per cent of the dogs poisoned with mercury bichloride, 45 per cent of the dogs poisoned with uranyl nitrate and only 22.2 per cent of the dogs poisoned with potassium dichromate.

Attempts to connect phosphatase with the deposition of calcium in the kidneys involves chemical theorizing that is of questionable application in these organs. Thus, Guttman and Guttman [16] have stated that hexose–phosphoric esters may be formed locally by the interaction of glycogen and inorganic phosphate and that these esters may then be acted on by phosphatase. But renal tissue is too poor in glycogen for this process to occur to any appreciable extent. Another possibility may be stated as follows: Wilmer [6] has described a somewhat theoretic mechanism by which dextrose in the glomerular filtrate is absorbed. According to this scheme the dextrose of the filtrate is phosphorylated by renal phosphorylase. The hexose ester thus formed is then split by phosphatase, and the freed dextrose is passed through the tubular epithelium to the tissue spaces and thence into the blood of the peritubular capillaries. Although the chemical agents used in our experiments do not completely inactivate phosphatase, their effect on phosphorylase is not known. If the latter enzyme is still active in kidneys damaged by these agents, the orderly process described by Wilmer could be markedly deranged as a result of the markedly disrupted structural pattern. The PO_4 ions liberated from the hexose esters by phosphatase might be precipitated by calcium ions in the glomerular filtrate that permeates the necrotic mass instead of being recombined with more dextrose. This theory, if proved correct, may explain the glycosuria observed in severe poisoning with mercury bichloride, uranyl nitrate and potassium dichromate, which we plan to discuss in another paper in this series.

A recent suggestion by Gomori [3] may apply to calcification in the kidneys under the conditions of our experiments. Ross [17] has shown that under certain conditions tissues may calcify far below the critical level of $(Ca^{++}) \times (PO_4^{---}) = 3.3 \times 10^{-6}$, required for the calcification of bone. "One possibility unexplored so far," said Gomori, "would be the presence of some protein, resulting in a higher local concentration of calcium ions at the same level of total calcium." If such a protein is present in the debris from the necrotic renal epithelium, with an adequate supply of PO_4 and CO_3 ions available in the glomerular filtrate the precipitation of calcium as phosphate and carbonate might occur. But this will not explain the quantitative differences in the calcium deposited in the kidneys of dogs poisoned with necrotizing doses of the three substances employed in these experiments until qualitative differences have been demonstrated in the protein content of the necrotic material produced by these chemical agents.

SUMMARY AND CONCLUSIONS

Necrosis of the kidneys of dogs induced by chemical agents is frequently accompanied by calcification. The amount of calcium deposited varies with the agent, the dosage and the time that elapses between the administration of the poison and the death of the animal. In a group of 28 dogs with comparable degrees of necrosis of the renal tubules, calcification occurred in the kidneys of 100 per cent of those poisoned with mercury bichloride, 45 per cent of those poisoned with uranyl nitrate and only 22 per cent of those poisoned with potassium dichromate.

Phosphatase was not a factor in the deposition of calcium in the kidneys of these dogs. On the contrary, calcium appeared to be deposited only in those tubules in which the epithelium had been so severely damaged that the phosphatase had been rendered inactive or destroyed.

Calcification was most abundant in that part of the proximal convoluted tubule which in dogs may normally contain fat. It is suggested, therefore, that the presence of fat may be a factor in the deposition of calcium in the kidneys under the conditions of these experiments.

15. Best, C. H., and Taylor, N. B.: Physiological Basis of Medical Practice, ed. 1, Baltimore, William Wood & Company, 1937, p. 626.

16. Guttman, A. B., and Guttman, E. B.: Proc. Soc. Exper. Biol. & Med. **48**:687, 1941.

17. Ross, B. D.: Proc. Soc. Exper. Biol. & Med. **45**:531, 1940.

OSTEOID SARCOMA OF THE BREAST: A COMPLICATION OF FIBROADENOMA

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We have recently had an opportunity to study the clinical and pathologic features of a primary epulis-like tumor of the breast containing osteoid tissue. A review of the literature impressed on us the rarity of this tumor and—a matter of more importance—the fact that it is a dangerous cancer. Because of these facts a report of our study seems desirable.

Investigation of the literature soon disclosed scattered reports of primary tumors of the breast containing not only osteoid tissue but also bone, cartilage and polymorphous cells. We studied all available reports, ninety-seven in number, and decided that for the purposes of this study we would select only such as described neoplasms similar to ours even though it was believed that all the tumors were related regardless of whether they contained bone or cartilage, neither or both.

On this basis 25 cases were assembled.[1] Though osteoid tissue was described in only 10 of these,[2] we decided to accept them all for study regardless of the presence or the absence of osteoid tissue, for the following reasons: first, because examination of our own material impressed on us how easily such a component might be overlooked or even altogether missed unless many sections were studied; second, because osteoid tissue was noted in Morton's [1w] tumor only after a committee had restudied his slides, and third, because Hartman and co-workers [1r] considered their three giant cell tumors of the breast to be identical despite the fact that they found osteoid tissue in only one of them. Our decision seemed further justified by the fact that osteoid tissue was demonstrated in the recurrent tumor in Holm's [1v] case 1 though it had not been noted in the primary tumor.

The first reported case acceptable because of microscopic study was contributed by Lancereaux [1a] in 1860; the last 2, by Holm [1v] in 1940. In all there were 25. Analysis of the cases revealed the following facts:

There was only 1 male in the entire group.

One patient, the youngest, was 27 years of age; 1 was 36 and 1 was 37; 1 was 46; 7 were between 50 and 59 years; 8 were between 60 and 68 years; 1 was 86.

The tumor occupied the right breast in 11 cases and the left in 8; in the remainder the position was not specified.

There was extreme variation in the time which elapsed between discovery of the tumor and medical attention. In 8 cases this period was short: three weeks, one month, two months, two months, three months, five months, six months and eight months. In 10 cases the period was long; in 1, one year; in 2, two years, in 2, ten years; in 2, twelve years; in 1, thirteen years, and in 1 each, nineteen, twenty and thirty years.

Consciousness of mass was the only symptom experienced by most of the patients; a few also felt pain.

The growth of the tumors of long duration was as a rule slow and was either progressive or periodic. In a few instances it tended to become

From St. Vincent's Hospital.

1. (*a*) Lancereaux, E.: Bull. Soc. anat. de Paris **35**:293, 1860. (*b*) Haward, W.: Tr. Clin. Soc. London **7**:106, 1874. (*c*) Stilling, H.: Deutsche Ztschr. f. Chir. **15**:247, 1881 (case 3). (*d*) Pillier: Bull. et mém. Soc. anat. de Paris **4**:552, 1890. (*e*) Noetzel, W.: Ein Beitrag zur Kenntnis der Fibroadenome der weiblichen Brustdrüse, Berlin, G. Schade, 1892. (*f*) Snow, H.: Tr. Path. Soc. London **46**:184, 1895. (*g*) Manz, O.: Beitr. z. klin. Chir. **13**:66, 1895. (*h*) Heuter, C., and Karrenstein: Virchows Arch. f. path. Anat. **183**:495, 1906. (*i*) Lorrain and Ménard: Bull. et mém. Soc. anat. de Paris **84**:103, 1909. (*j*) Desmarest, R., and Masson: ibid. **87**:221, 1912. (*k*) Orlandi, N.: Osp. maggiore **1**:519, 1913. (*l*) Moure, P., and de Jong: Bull. et mém. Soc. anat. de Paris **89**:32, 1914. (*m*) Jessup, S. D.: Proc. New York Path. Soc. **19**:117, 1919. (*n*) Fry, J. B.: J. Path. & Bact. **30**:528, 1927. (*o*) Edelmann, H.: Beitr. z. path. Anat. u. z. allg. Path. **78**:618, 1927. (*p*) Wellbrock, W. L.: Ann. Surg. **90**:154, 1929. (*q*) Caligaris, E.: Cancro **3**:226, 1933. (*r*) Hartman, H.; Bertrand, T.; Fontaine, and Guérin, P.: Bull. Assoc. franç. p. l'étude du cancer **22**:378, 1933. (*s*) Rose, J.: Deutsche Ztschr. f. Chir. **246**:151, 1936. (*t*) Guérin, P.: Bull. Assoc. franç. p. l'étude du cancer **25**:326, 1936. (*u*) Di Stefano, R.: Tumori **23**:482, 1937. (*v*) Holm, E.: Acta path. et microbiol. Scandinav. **17**:506, 1940. (*w*) Morton, C. A.: Tr. Path. Soc. London **54**:327, 1904.

2. Stilling.[1c] Pillier.[1d] Heuter and Karrenstein.[1h] Orlandi.[1k] Fry.[1n] Edelmann.[1o] Hartman and others,[1r] case 2. Guérin.[1t] Holm,[1v] 2 cases.

accelerated in the later stages. The growth of neoplasms of recent discovery, on the other hand, tended to be rapid from incipiency.

Alteration of physical characteristics occurred in some of the tumors. A few, which at first were firm, softened to become cystic. Five, originally movable, became fixed to the overlying skin and finally ulcerated through it. The skin overlying many of the tumors thinned and assumed a violaceous color. Superficial veins of some appeared prominent.

The size of the tumors varied: "first-sized," "size of a baby's head," "size of an adult's head."

Lymph nodes were palpable in many cases. In only 1 case was this subsequently proved to be due to tumor involvement.

The treatment employed was excision in 5 cases, mastectomy in 7 cases, radical mastectomy in 4 cases, excision and subsequent mastectomy in 3 cases, and surgical removal supplemented by radium treatment in 2 cases. None of these treatments was successful. Local recurrence was observed early—as a rule, two to four months after removal of the breast. Sixteen of the 25 patients on whom there were follow-up data were dead before twelve months had elapsed regardless of the extent of surgical treatment.

Metastases, chiefly in the lungs, were clinically evident in many cases. Unfortunately, autopsy was performed in only 3 cases [3] ; in 2 of these metastases were found.[4]

PATHOLOGY

As a rule the tumors were demarcated from the surrounding breast tissue but not encapsulated. The smallest tumor was "pigeon egg–sized" and the largest 30 by 28 by 18 cm. Their color varied, some being gray and others brownish, gray-red or mottled yellow. A few of the tumors were firm, though most of them were either soft or friable. Many were cystic, with single or multiple cavities. These varied in size and usually contained broken-down, hemorrhagic material. In many cases their lining was obviously tumor.

The tumors presented a characteristic histologic picture. Though each differed from the others in minor respects, all contained spindle cells, polymorphous cells and multinuclear giant cells. The latter were most striking, having as many as sixty or more nuclei. In many instances the cells were closely packed. Fibrous stroma varied in amount in different tumors. Many of the cysts represented areas of degenerated tumor; others were derived from dilated lymph channels or blood sinuses. In 10 cases osteoid tissue was noted, making its appearance as trabeculae, plaques or small irregular islands with indented outlines. Often giant cells lay within these indentations, and some of the osteoid masses contained lacunas enclosing cells resembling osteoblasts. In 2 instances the osteoid tissue was calcified.

Another interesting feature, noted microscopically in 10 cases, was the occurrence of fibroadenoma either as an integral part of the tumor or as a growth adjacent to it. Stilling [1e] discovered a fibroadenoma 2 to 3 cm. in diameter adjacent to the main growth. Pillier [1d] discovered two tumors, the first pure fibroadenoma, the second fibroadenoma containing areas of giant cell sarcoma, myxomatous connective tissue, irregular plaques of osteoid tissue and epithelial cysts. At the periphery of the tumor described by Heuter and Karrenstein [1h] a fibroadenoma was observed. The growth in the case reported by Desmarest and Masson [1j] appeared to be fibroadenoma containing large giant cells having numerous centrally located nuclei and a "sarcomatous stroma." Surrounding it were four small, noncomplicated fibroadenomas. Two closely adjacent tumors were observed by Fry [1n] in the case he studied, one a typical osteoid giant cell sarcoma, the other a fibroadenoma containing osteoid tissue surrounded by giant cells with one or two nuclei. The patient observed by Hartman and his associates [1r] had a fibroadenoma containing multinuclear giant cells. Eight years later there appeared in this patient a tumor having the histologic character of giant cell sarcoma. Remnants within the tumor studied by Guérin were fibroadenoma. The tumor of Di Stefano [1s] appeared to be fibroadenoma, portions of which contained spindle cells and numerous multinucleated giant cells. Holm [1v] says of his first case, "a small part of the tumor was indistinguishable from fibroadenoma", and of case 2, "examination reveals fibroadenoma of the intracanalicular variety, in several areas of which there is a tumor tissue structure like a giant cell tumor of bone."

Autopsies were made on 3 of the 25 patients. One of these had a metastasis in the heart,[1e] the second [11] had a metastasis in the lungs, and the third [1g] was completely free of metastasis. In 5 instances [5] biopsy specimens of the recurrent tumor were procured. Microscopic examination of both the recurrent and the metastatic tumors showed

3. Stilling.[1e] Manz.[1g] Moure and de Jong.[11]

4. Stilling.[1e] Moure and de Jong.[11]

5. Stilling.[1e] Heuter and Karrenstein.[1h] Moure and de Jong.[11] Wellbrock.[1p] Holm.[1v]

osteoid tissue and the same cellular components, including the giant cells, which had been observed in the parent tumor. Very interesting is the fact that Holm did not find osteoid tissue in the specimen removed at the first operation but did find it in the recurrent growth.

In only 1 case was a metastasis in a lymph node demonstrated.[1m]

REPORT OF A CASE

V. D., a white woman, single, aged 48 years, entered St. Vincent's Hospital, Feb. 15, 1944, because of a swollen, painful right breast. Her past history was essentially without significance in regard to the neoplasm. The last menstrual cycle had occurred four years previously.

Early in December 1943 she became aware of a mass in the right breast, which grew rapidly and became painful. Palpation of the breast revealed a grapefruit-sized indurated mass, which felt warm and in one portion fluctuant. On February 16 an incision was made through the fluctuant area, releasing hemorrhagic fluid and detritus. After exploring the cavity from which the fluid was released, the operator concluded that a neoplasm was present and thereupon proceeded to do a simple mastectomy.

The postoperative course was uneventful, and the patient left the hospital March 2. Two months later a nodule appeared adjacent to the mastectomy scar. Between May 22 and August 14 a total dose of 2,700 roentgens (r) was applied to this nodule without visible effect. On July 27 a roentgen study of the chest disclosed sharply demarcated nodules in both lung fields. A total of 1,000 r was therefore applied to the posterior parts of the lungs during a period extending from August 4 to August 14. This produced no sign of regression of the nodules. In fact, a picture taken on September 29 showed them to have increased in size and indicated the development of new ones.

The patient died November 6, evidently from metastasis. There was no autopsy.

Pathologic Report.—The specimen was a breast, the skin of which was incised. On section one found within it a large, circumscribed, nonencapsulated, firm, gray tumor mass measuring 8 by 5 by 8 cm., extending from the skin to the deep fascia but not invading either. About one third of the tumor was occupied by an irregular cavity (fig. 1), the contents of which had been evacuated. Elsewhere there were other spaces resembling large blood vessels, measuring from 3 to 10 mm. in diameter and lined by thick fibrous membrane. The remainder of the breast was fatty.

The entire tumor was cut transversely so that each block contained the whole thickness of the tumor. Large sections were prepared from each and stained with hematoxylin and eosin, with Masson's trichrome stain as modified by Larson and Levin[6] and with the Foot and Foot[7] reticulum stain.

Study of these sections revealed a very cellular tumor containing sinusoids, collagen and small scattered islands of osteoid tissue. While many of the tumor cells were spindle in type, with oval nuclei (fig. 2), most were irregular in size and in shape (fig. 3). One cell in particular was conspicuous because of its large size and its many nuclei (fig. 4). All of these cells—spindle, polymorphous and giant cells—were variously arranged. Thus, in places the spindle cells predominated and were grouped in intertwining, whorling bundles, presenting the typical picture of spindle cell sarcoma (fig. 2). In other areas giant cells were observed in great profusion, reminding one of an epulis, and in still other fields one found all of them intimately intermixed. Blood vessels were numerous and sinusoidal (figs. 1, 3 and 4). Many were widely dilated, serpentine in shape and lined by flat endothelial cells. Other channels appeared as broad, blood-filled pools measuring up to 0.5 mm. in diameter. The supporting framework of the tumor consisted of a rich network of fibrils as revealed by the Foot and Foot silver nitrate method. In places these fibrils formed meshes so small that single cells only were enclosed between the fibrils. Usually the meshes were larger and embraced small clusters of five to six cells. Elastic tissue was absent. Much of the surface of the large cavity (fig. 1) was covered directly by tumor cells. The smaller cavities described grossly were lined with a thick layer of collagen, edematous in some places, dense and hyalinized in others.

Other spaces not detected with the naked eye were also observed, and these were filled with blood and surrounded and lined by tumor cells. Some of these spaces could still be identified as blood channels, indicating invasion and replacement of the endothelial lining by the tumor. Within the lumens of some of these broken-down vascular channels giant cells were observed, free.

One interesting finding was osteoid tissue (fig. 5), present as small irregular islands scattered throughout the tumor. It appeared as a deeply eosinophilic homogeneous hyalinized substance interrupted by spaces enclosing small cells. None of the osteoid tissue was calcified. While some of the osteoid tissue was observed in cell-rich areas, other masses were found in areas less rich in cells, where reticulum was more conspicuous.

In several places at the periphery but still within the tumor small lactiferous ducts were present. In another area an acorn-sized intracanalicular fibroadenoma (fig. 6) was found enclosed within the wide duct in which it had originated. Portions of the fibroadenoma contained small masses of osteoid tissue. In several areas portions of the fibroadenoma were replaced by giant cell tumor.

COMMENT

The clinical behavior and the pathologic features of the neoplasm just presented are typical of similar tumors described in the literature. Our patient suffered from a rapidly growing neoplasm which recurred three months after it was removed and which caused death in ten months despite intense roentgen therapy.

Though regional lymph nodes remained free, the lungs became seeded with metastases. One of the outstanding gross characteristics of this type of tumor was present in ours, namely, a large cyst filled with necrotic, hemorrhagic debris. The cyst had evidently developed through a process of necrosis and liquefaction of a portion of the tumor. Hemorrhage represented a late complication.

6. Larson, C. P., and Levin, E. J.: Arch. Path. **29:** 272, 1940.

7. Foot, N. C., and Foot, E. B.: Am. J. Path. **8:** 245, 1932.

The tumor itself was abundantly cellular, and the cells were polymorphic. Outstanding among these were numerous multinuclear giant cells. Another important finding in our case was osteoid tissue, described in only 9 of the 25 cases assembled from the literature. A study of sections prepared by the Foot and Foot silver nitrate method and by a trichrome technic satisfied us that the origin of the osteoid tissue was traceable to condensed masses of collagen and reticulum.

The histologic nature of the recurrent tumor and of the metastases in the lungs is uncertain in our case because postmortem examination was lacking. However, study of other reported cases

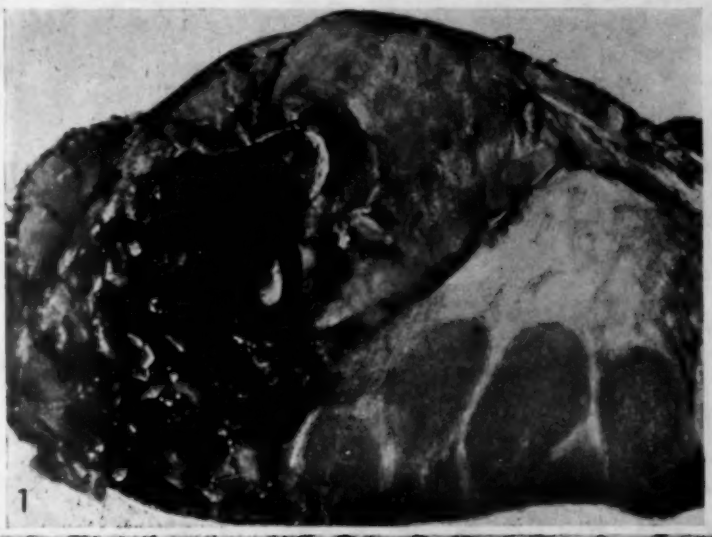
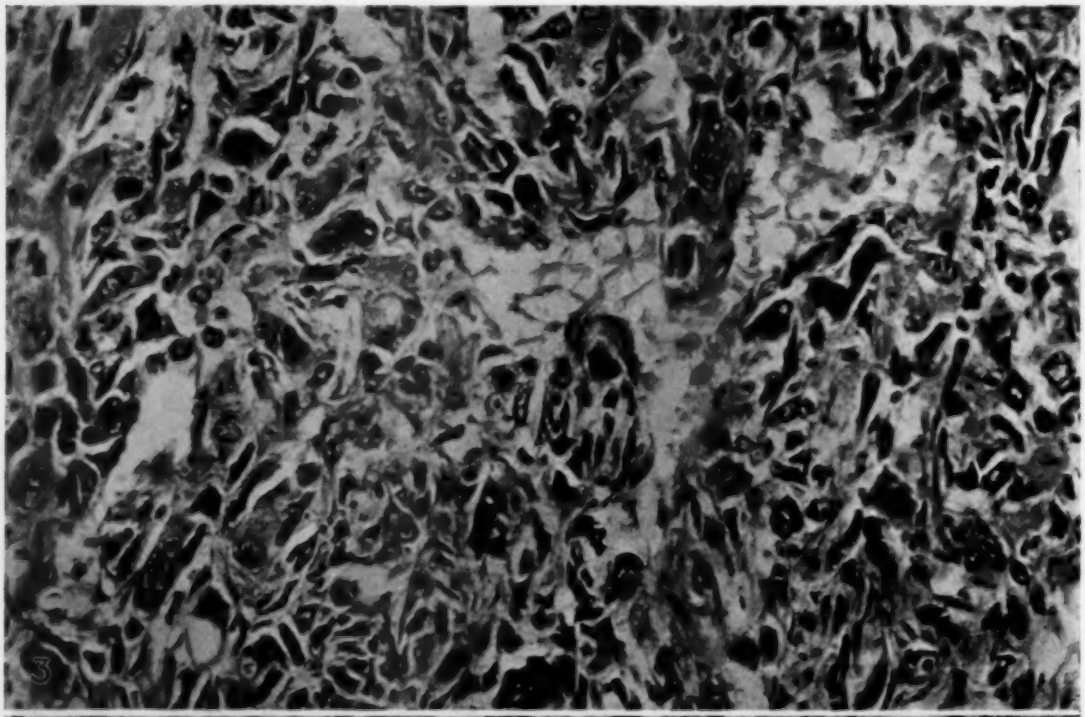


Fig. 1.—Cross section of the breast. Note that nearly half of the organ is occupied by a tumor, the periphery of which is sharply demarcated from the normal portion of the breast. The large cavity was filled with old blood and necrotic debris.

Fig. 2.—Tumor tissue; low power magnification; hematoxylin and eosin stain. In the area photographed the tumor cells were spindle shaped and arranged in bundles. Note the presence of sinusoids lined by flat endothelial cells.

indicates that the parent tumor is faithfully reproduced by its local and distant offspring. In 10 of the cases reported in the literature fibroadenoma was observed in intimate association with the giant cell tumor. In some cases there were only remnants of fibroadenoma; in a few others the main tumor was fibroadenoma, and the osteoid and giant cell phase of the growth was observed



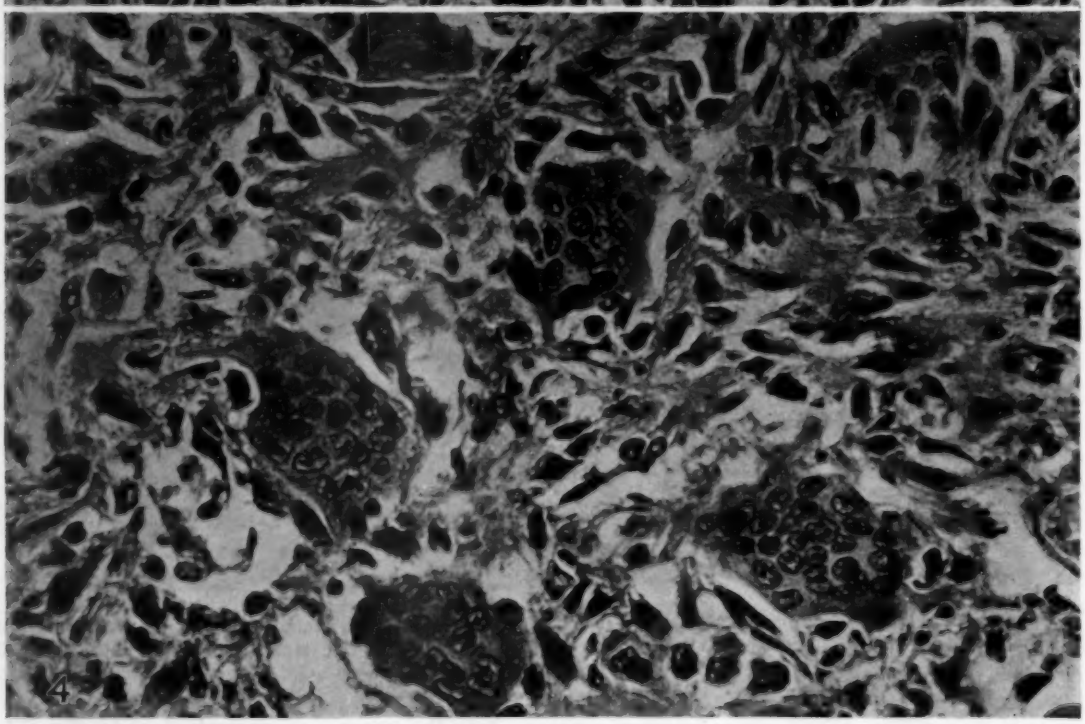
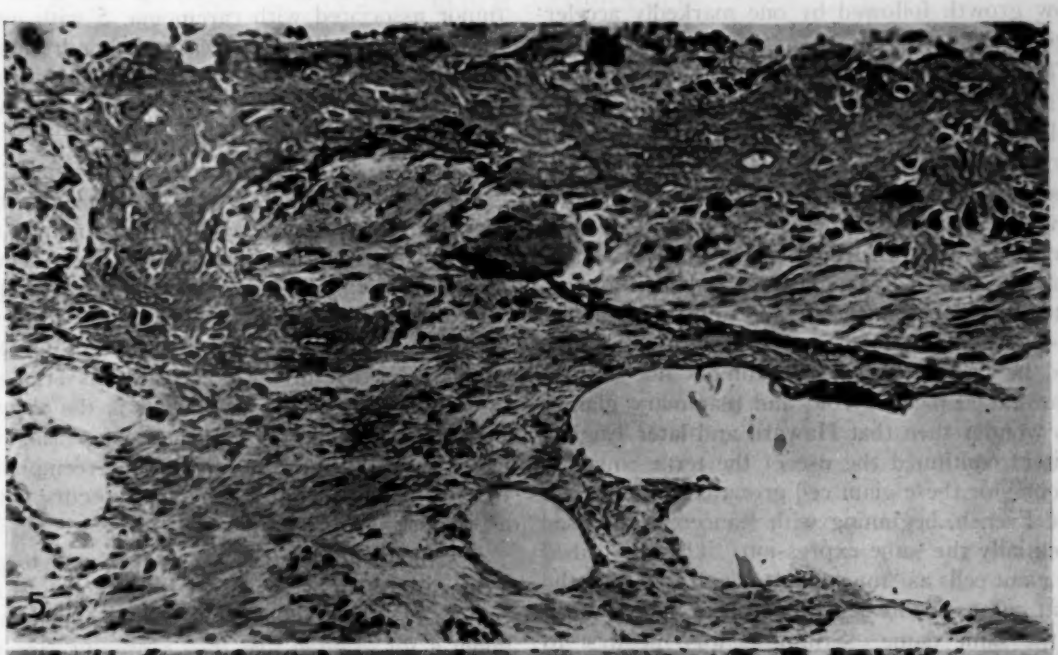


Fig. 3.—Tumor tissue; high power magnification; hematoxylin and eosin stain. In this section, the more pleomorphic cells of the tumor are observed, and the variation in size, shape and staining are well illustrated.

Fig. 4.—Tumor tissue; high power magnification; hematoxylin and eosin stain. Note the multinuclear giant cells. These constitute a striking component of the tumor.

developing within it. In our case also a small area of fibroadenoma was found. It was surrounded and invaded by the giant cell portion of the tumor. The fibroadenoma could easily have been missed had we failed to cut all the blocks which we did, and also if by misfortune the blocks containing the fibroadenoma had been embedded on the opposite surface. Hence failure of other writers to describe this tumor cannot be interpreted as indicating its absence unless one knows that a deliberate attempt was made to find it.



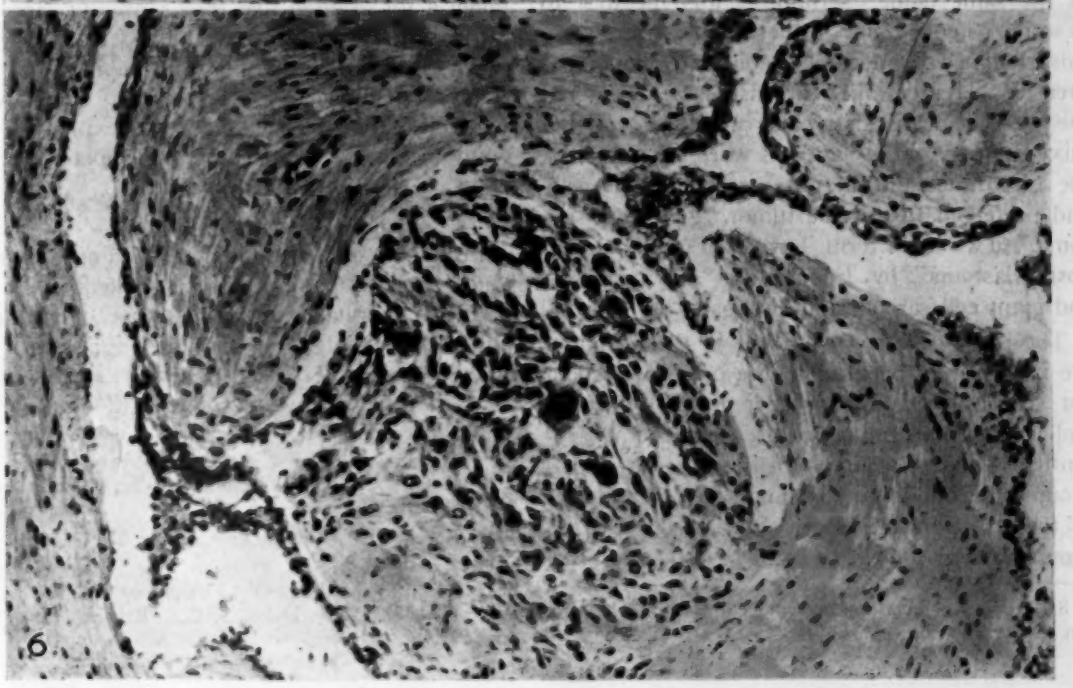


Fig. 5.—An area containing osteoid tissue; low power magnification; hematoxylin and eosin stain.

Fig. 6.—Fibroadenoma found in the substance of the osteoid giant cell tumor; low power magnification; hematoxylin and eosin stain. In the center a nest of malignant cells is observed. Though at this level the cells appear isolated, at deeper levels they are seen to be continuous with similar cells from the main tumor.

Stilling [1c] was the first to propose that the giant cell tumor arose in a fibroadenoma. Many have since agreed with him, including ourselves.

Granting this to be a fact, namely, that a stage of uncomplicated fibroadenoma precedes the cancerous phase, one can see a meaning to the clinical awareness of the tumors for twelve, nineteen and even thirty years. It also explains the period of slow growth followed by one markedly accelerated.

The earliest writers recognized the resemblance of these tumors of the breast to giant cell tumors of bone, a fact reflected in the nomenclature used to classify the growth. Thus Paget [8] in 1863 proposed the term "myeloid tumor" for a bone tumor in which the most characteristic constituents were "peculiar, many-nucleated corpuscles which have been recognized by Köllicker and Robin as constituents of the marrow and diploe of bones, especially in the foetus and in early life." Paget said he had observed such tumors not only in bone but also in thyroid and mammary glands. No wonder then that Haward and later English writers continued the use of the term "myeloid tumor" for these giant cell growths of the breast. The French, beginning with Lancereaux,[1a] used essentially the same expression. They described the giant cells as "myeloplaxes" and regarded the giant cell as identical with the bone marrow cell of the same name. Stilling [1c] was the first to describe osteoid tissue in these tumors, and from this stemmed the term "osteoid sarcoma of the breast." Heuter and Karrenstein [1h] also noted the presence of this tissue and called the growth a mixed tumor. Many authors were impressed by the appearance and the number of the giant cells and preferred to call the tumor "giant cell sarcoma." Two other terms have been used, namely, "osteoclastoma" by Fry [1n] and "polymorphous and giant cell sarcoma" by Caligaris.[1q]

The giant cells so prominent in these tumors are not entirely peculiar to the osteoid sarcoma but are observed also in other types of osseous and cartilaginous tumors of the mamma. We recently studied all available cases of the latter two groups and found many instances in which identical types of giant cells were observed. These are summarized as follows: (1) 21 cases of chondrosarcoma, 4 with giant cells; (2) 8 cases of osteochondroma, 3 with giant cells; (3) 12 cases of osteochondrosarcoma, 8 with giant cells; (4) 5 cases of osteosarcoma, 3 with giant cells; (5) 9 cases of ossifying, chondrifying and giant cell tumor associated with carcinoma, 5 with multinuclear giant cells; (6) 1 case of chondrolipoma, with no giant cells; (7) 7 cases of chondroma, with no giant cells; (8) 1 case of teratoma, with no giant cells.

Efforts to explain the origin of the tumor have given rise to a number of theories. Early writers, Arnold [9] and Lecène,[10] advanced the opinion that it developed from aberrant skeletal tissue sequestrated from the thorax. Another group [11] held instead that it had its genesis in misplaced pleuripotential embryonic cells—teratoma in other words. Neither of these theories has been sustained. More widely supported is the view of Stilling [1c] ascribing the tumor to metaplasia. We agree with this view and would only reemphasize that in many cases the metaplasia occurs within a preexisting fibroadenoma.

It is not within the scope of this paper to give a full discussion of the nature of the cells of these tumors. Most writers, ourselves included, have taken it for granted that they represent sarcoma. However, it should be mentioned that in 1929 when Jessup [1m] presented his case as an instance of giant cell sarcoma there was one dissenting voice, that of Ewing,[1m] who expressed the opinion that the tumor was an anaplastic carcinoma.

SUMMARY

Study of a case of osteoid sarcoma of the breast together with 25 other cases culled from the literature emphasizes that this type of tumor is undoubtedly only one member of a family of chondrogenic and osteogenic tumors of the mamma. The evidence indicates that it arises from fibroadenoma.

8. Paget, J.: Lectures on Surgical Pathology, London, Longman [and others], 1863, p. 519.

9. Arnold, S. K.: Virchows Arch. f. path. Anat. **148**:449, 1897.

10. Lecène, P.: Rev. de chir., Paris **33**:434, 1906.

11. Nadal, P.: Bull. et mém. Soc. anat. de Paris **85**:616, 1910. Kreibig, W.: Virchows Arch. f. path. Anat. **256**:42, 1925. Dyke, S. C.: Brit. J. Surg. **14**: 323, 1926.

SOLITARY GIANT FOLLICULAR LYMPHOMA OF THE VERMIFORM APPENDIX

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The vermiform appendix is an organ which is relatively free from tumor growth. Neoplasms which do arise in this locality are usually carcinoids, and only in rare instances is any other type of tumor growth represented. Among the extremely rare appendical neoplasms are the various types of sarcoma. In 1921 Goldstein [1] found only 17 authentic cases of primary sarcoma of the appendix recorded in the literature, and in 1926 Friend [2] would admit only 2 additional cases. The reports of appendical sarcoma have in most instances (72 per cent) [3] represented one of the several varieties of cancerous lymphoma. In the majority of instances the tumor has been lymphosarcoma, and it did not differ histologically from tumors of this type arising elsewhere in the body.

A patient suffering from cancerous lymphoma usually presents multiple tumors, and only in rare instances is a solitary lymphoma found. Primary solitary lymphoid tumors have been described in various organs of the body, including all of the anatomic divisions of the gastrointestinal tract. Among 3,132 cases of cancerous involvement of the gastrointestinal tract coming under their observation, Warren and Lulenski [4] were able to find only 28 cases of solitary lymphoid tumor (0.9 per cent). In 14 of these the tumor was located in the stomach; in 8, in the large intestine, and in 6, in the small intestine. Thirteen of them were classified as instances of Hodgkin's disease, 10 as instances of lymphosarcoma, and 5 as cases of cancerous lymphoma. Although the appendix is rarely the site of a solitary cancerous tumor of lymphoid tissue, such a neoplasm, when it does arise, probably does not differ fundamentally from primary cancerous lymphoma in other portions of the gastrointestinal tract.

From the Division of Pathology of the Department of Pathology and Bacteriology, Bowman Gray School of Medicine of Wake Forest College, and the North Carolina Baptist Hospital.

1. Goldstein, H. I.: Am. J. M. Sc. **161**:870, 1921.
2. Friend, E.: Illinois M. J. **50**:55, 1926.
3. Bizard, G.; Driessens, J., and Malatray, H.: Rev. de chir. **57**:195, 1938.
4. Warren, S., and Lulenski, C. R.: Ann. Surg. **115**:1, 1942.

The terminology employed in various classifications of the diseases of lymphoid tissue is admittedly confusing, and in many instances a proper interpretation of the morphologic picture is extremely difficult. This situation is largely the result of incomplete knowledge concerning the origin of the cells which make up this tissue. The extensive distribution of lymphoid tissue in areas of potential irritation and its tendency to show a proliferative response to various irritants are also factors.

The problem of differentiating normal from abnormal proliferative states in the appendix is made more difficult by the wide variations in the distribution and the behavior of the lymphoid tissue in this organ and also by the qualitative and quantitative variations incident to age. Distinguishing between hyperplasia and neoplasia in a given instance is sometimes extremely difficult. Before lymphoid tumors of the appendix and the possible transformation of hyperplastic into neoplastic states are discussed, it seems desirable to review briefly the available information regarding the normal variations of the lymphoid tissue in this organ and the behavior of this tissue under the influence of various types of stimuli.

THE AMOUNT AND THE CHARACTER OF THE LYMPHOID TISSUE IN THE NORMAL HUMAN APPENDIX AT DIFFERENT AGE PERIODS

The lymphoid tissue in the normal appendix has been studied by several investigators. The most complete investigation, however, was carried out by Hwang and Krumbhaar,[5] who examined a large series of appendixes from persons meeting violent deaths from various causes. These studies showed that the lymphoid tissue in the human appendix reaches its maximum during the first decade and after this period steadily decreases throughout life. It is the predominant tissue in the appendix during the first two decades of life and in some instances may constitute nearly 50 per cent of the total substance of that organ. Hwang and Krumbhaar

5. Hwang, J. M. S., and Krumbhaar, E. B.: Am. J. M. Sc. **199**:75, 1940.

pointed out further that only during the first and second decades of life and under normal conditions do the pale centers of the lymph follicles contribute materially to the total amount of lymphoid tissue in the organ. These authors concluded that the pale centers in children probably represent "germinal centers" which are lymphoblastic and actively concerned with the normal lymphoid hyperplasia which characterizes this period. It was concluded also that under normal conditions persons in the older age groups do not possess "germinal centers" in their appendixes and that when these are present they represent "reaction centers" in response to pathologic stimuli. While it is unlikely that conditions of lymphoid hyperplasia in normal appendixes have been mistaken for neoplasms, they have, in all probability, been erroneously diagnosed as "lymphoid appendicitis." This condition has been incriminated by some as a precursor of neoplasia.

LYMPHOID HYPERPLASIA OF THE APPENDIX

Lymphoid hyperplasia of the appendix has been described by numerous investigators. Many have considered the condition a manifestation of "status lymphaticus," [6] while others have considered it a separate clinical and pathologic entity.[7]

Interest in the condition has centered chiefly around the possibility of its being a type of acute or recurrent appendicitis. A possible relationship between hyperplasia and neoplasia in the lymphatic tissues wherever it is found has also been suggested.

The majority of the patients in whom it has been reported have been females in the second decade of life. The most common complaint is a cramplike pain in the right lower quadrant of the abdomen which does not radiate; tenderness is either not present in this area or is only moderate in degree. Nausea is experienced in some cases and at times is associated with vomiting.

The total and differential leukocyte counts are usually normal, although in some cases there is slight leukocytosis.

Pathologically, the appendixes of the patients have been described as characterized by an absolute and a relative increase in the amount of lymphoid tissue to such a degree that it constituted more than one half of the thickness of the wall. In addition to the quantitative change in the lymphoid tissue, hyperplasia of the lymph follicles has been noted.[7] Symmers and Greenberg [8] divided their cases into three groups: (1) those characterized by hyperplasia of the lymphoid tissue alone, (2) those in which degeneration and sclerosis were added to the hyperplastic process and (3) those in which the appendix was reduced in size and the lymphoid tissue was almost completely replaced by connective tissue. Of importance is the fact that all of these changes are not infrequently seen in appendixes removed from persons who have not suffered from appendical symptoms. Gray and Heifetz,[7] although recognizing the condition as a clinical and pathologic entity, were careful to point out that there were no changes in their material which could not be duplicated in normal secondary lymph nodules and that the alterations produced were more indicative of an "active physiological process than of a toxic or destructive lesion."

A study of the reported cases of lymphoid hyperplasia of the appendix reveals that they have for the most part been arbitrarily selected and that no attempt has been made to control the various series carefully. Since all of the morphologic features described in this condition have been seen repeatedly in persons who gave no history of pain or other symptoms referable to the appendix, lymphoid hyperplasia of the appendix cannot at present be unreservedly accepted as a disease entity. A critical review of the subject, together with a clinicopathologic study utilizing controls and employing methods similar to those of Hwang and Krumbhaar, is indicated.

ABNORMAL ACCUMULATIONS OF LYMPH FOLLICLES IN THE APPENDIX AND IN OTHER PORTIONS OF THE DIGESTIVE TRACT

Localized masses of lymphoid tissue in the digestive tract have been noted by several observers, and in rare instances the accumulations were confined to the appendix. The condition is characterized by an abundance of lymph follicles possessing prominent pale centers and distributed throughout the wall of the organ. In some instances follicles have also been seen in the mesentery.

Stout [9] has described 3 cases in which localized hyperplasia of lymphoid tissue occurring in the cecum and in the appendix was not associated with status lymphaticus or with evidence of lymphoid hyperplasia in other portions of the gastrointestinal tract. In Stout's first case the lymphoid mass was located in the ileocecal valve; in the second case it occurred at the

6. Symmers, D.: Am. J. Surg. **26**:7, 1934.

7. Gray, S. H., and Heifetz, C. J.: Arch. Surg. **35**:887, 1937.

8. Symmers, D., and Greenberg, M.: J. A. M. A. **72**:468, 1919.

9. Stout, A. P.: Am. J. Dis. Child. **34**:797, 1927.

junction of the appendix and the cecum; in the third case the tip of the appendix alone was involved. Wilhelm and Delval [10] and Lehmann [11] have reported similar hyperplastic deposits in the tip of the appendix. Recently Gruenwald [12] has reported 5 instances of abnormal lymphoid accumulations in the digestive tract, in 2 of which they were located in the appendix. In each instance the microscopic picture was that of simple lymphoid hyperplasia.

The histogenesis of these lesions is not clear. Lehmann [11] and Knoflach [13] considered them to be congenital in origin, while Gruenwald [12] suggested a predisposition to such growths was necessary in addition to the stimulation of the tissue involved. The development of new methods for investigating the more fundamental problems of abnormal growth is necessary for clarification of this and related questions.

GIANT FOLLICULAR LYMPHOMA (GIANT FOLLICULAR LYMPHADENOPATHY, GIANT FOLLICULAR HYPERPLASIA, BRILL'S DISEASE, BRILL-SYMMERS DISEASE)

This condition, described originally by Brill, Baehr and Rosenthal [14] and later more elaborately by Symmers,[15] is regarded as a generalized disease of lymphoid tissue characterized by dimensional and numerical hyperplasia of the lymph follicles which results in replacement of the normal structure of lymphoid organs by proliferating lymphoid tissue. In some of the cases reported the spleen has shown massive enlargement, while in others it was of normal size. Although the disease is considered a generalized malady of lymphoid tissue, a form has been described in which the spleen seems to be the primary site of the lymphoid hyperplasia, the lymph nodes and other lymphoid structures being apparently free from the disease or showing only minimal involvement. Symmers [16] expressed the opinion, however, that "in the absence of evidence revealed at necropsy, the existence of preponderant or 'primary' giant follicular lymphadenopathy of the spleen cannot be affirmed."

Recently Symmers [16] described 9 appendixes characterized by marked hyperplasia of the lymph follicles, which encroached on the lumen of the organ and almost completely replaced the mucosa and the muscularis, without clinical evidence of lymphoid disease elsewhere in the body. The microscopic changes were similar to those seen in generalized giant follicular lymphoma. Since all of the other types of cancerous lymphoma occasionally occur as solitary tumors, it would not be surprising to find this relatively benign form arising in a similar manner.

THE TRANSFORMATION OF GIANT FOLLICULAR LYMPHOMA INTO INVASIVE TUMOR

Although giant follicular lymphoma was originally considered a manifestation of benign lymphoid hyperplasia,[17] there is little doubt about its ultimate cancerous nature.[18] In some cases, however, the lymphoid enlargements have decreased in size, while in others they have temporarily disappeared. It is probable that the disease can be transformed into other types of cancerous lymphoma, and recently Symmers described its transformation into a tumor which he designated as "polymorphous cell sarcoma." [19] He stated that as long as the hyperplastic collections of cells remain within the follicles the condition should be classified as giant follicular lymphadenopathy, but that a rupture of the follicles with the cells infiltrating the surrounding tissues represents a direct transformation of giant follicular lymphadenopathy into "polymorphous cell sarcoma." He objected to the term "lymphosarcoma" on the ground that conclusive evidence establishing the lymphocyte as a unit of growth had not been produced. He preferred instead the descriptive term "polymorphous cell sarcoma" since the invasive cells are of three distinct morphologic types. These cells will be described more fully in the following report of cases.

The purpose of this paper is to describe 3 appendixes, all of which showed (1) lymphoid hyperplasia, (2) giant follicular hyperplasia and (3) direct transformation of giant follicular lymphoma into the "polymorphous cell sarcoma" of Symmers.

REPORT OF CASES

CASE 1.—A 33 year old Negro woman consulted Dr. G. F. Busby, Salisbury, N. C., May 31, 1943, complaining of recurrent attacks of pain in the right lower quadrant of the abdomen of five years' duration. These attacks were associated with nausea and vomiting. On two occasions they were so severe that she was advised by her physician to have her appendix removed. Five days before she came to Dr. Busby she experienced an attack which was so severe that it confined her to bed and necessitated her admission to the hospital.

10. Wilhelm, A., and Delval, C.: Bull. et mém. Soc. anat. de Paris **85**:771, 1910.
11. Lehmann, H.: Deutsche Ztschr. f. Chir. **190**:391, 1925.
12. Gruenwald, P.: Am. J. M. Sc. **203**:823, 1942.
13. Knoflach, J. C.: Wien. med. Wchnschr. **40**:876, 1927.
14. Brill, N. E.; Baehr, G., and Rosenthal, N.: J. A. M. A. **84**:668, 1925.
15. Symmers, D.: Arch. Path. **3**:816, 1927.
16. Symmers, D.: Arch. Int. Med. **74**:163, 1944.
17. Brill and others.[14] Symmers.[15]
18. Gall, E. A., and Mallory, T. B.: Am. J. Path. **18**:381, 1942.
19. Symmers, D.: Arch. Path. **34**:385, 1942.

Examination revealed tenderness and slight rigidity in the right lower quadrant. There was no evidence of lymphadenopathy or splenomegaly, and no other significant physical findings were noted. The serologic examination gave positive findings but the results of the laboratory studies were otherwise within normal limits.

The appendix was removed on June 4 without difficulty. It measured 8 cm. in length and approximately 8 mm. in diameter except in the distal third. In this area there was a bulbous dilatation which measured 1.5 cm. in diameter. The serosa of the organ was smooth and glistening, and on section the lumen was found to be patent except in the distal third, where it was completely obliterated by a grayish white tissue forming a definite tumor mass.

Microscopic examination of sections removed from the proximal and middle thirds showed the serous, muscular and fibrous coats to be of normal thickness (*B* in fig. 1). The mucosa contained numerous spherical or oval lymphatic nodules having pale centers, composed for the most part of medium-sized lymphocytes in an active state of proliferation. Mitotic figures were numerous, and occasionally a reticulum cell or a macrophage was seen. These pale centers were surrounded by a distinct rim of small lymphocytes, and in no instance was there evidence that the cells of the pale centers had infiltrated into the surrounding tissues. Sections through the distal end of the organ showed that the greater part had been invaded by a tumor which was distinctly lymphoid in character (fig. 1 *A*). The mucosa had been completely replaced by tumor tissue, and there was extensive infiltration of the fibrous and muscular coats (fig. 2 *A*). The greater portion of these coats had been destroyed, and an occasional lymphatic nodule was seen in the mesentery. Most of the tumor was composed of large lymphatic nodules, which presented many bizarre shapes. The pale centers were irregular and communicated freely with one another (fig. 2 *B*). In some instances there was no sharp line of demarcation between them, and infiltration of the surrounding tissues was obvious. The pale centers were composed of large hypochromatic cells, smaller and more chromatic cells, and cells which resembled medium-sized lymphocytes (fig. 2 *C* and *D*). These cells have been thoroughly described and illustrated in Symmers' papers.[20]

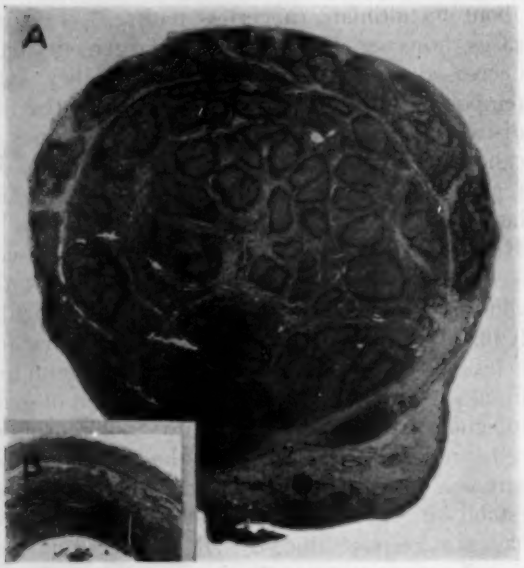

Fig. 1.—*A*, section from the distal end of the appendix in case 1; × 8. The normal structure of the organ has been almost completely replaced by large lymphatic nodules. Several of the large pale centers communicate with one another. *B*, section from the middle third of the appendix; × 8. All the coats are of normal thickness. The lymphatic nodules are hyperplastic but are much smaller than those seen in *A*. Note also that they are sharply demarcated from the surrounding tissues.

Case 2.—A white schoolboy aged 12 consulted Dr. J. G. Ramsay, Washington, N. C., Feb. 14, 1944, complaining of abdominal pain of twenty-four hours' duration. He stated that the pain was generalized at the onset but had gradually settled in the right lower quadrant. He had had slight nausea but no vomiting. His past medical history revealed that on three occasions he had suffered from identical attacks. Examination elicited tenderness in this area but no rigidity. The spleen was not palpable, and lymphadenopathy was absent. The urinalysis gave negative results, and the white blood cell count was 12,400. A differential count was not made at this time.

At operation the appendix was found to be bulbous at the tip; it was attached to the ileum for a distance of 1 inch (2.5 cm.). Many large glands were seen in the mesentery. The spleen was normal in size.

Sections prepared from the proximal and middle thirds of the appendix showed all of the coats to be greatly thickened. The serous, muscular and submucosal coats were thickened by fibrous tissue and infiltrated by large numbers of eosinophils, which in many instances were so numerous that they formed solid sheets of cells. The lymphoid tissue was abundant and was confined to the mucosa. Numerous large lymphatic nodules were present, which had prominent pale centers and were surrounded by rather dense accumulations of lymphocytes. The pale centers were composed primarily of medium-sized lymphocytes, which displayed numerous mitotic figures and were in an active state of lymphopoiesis. A few reticulum cells and an occasional macrophage were seen. All the lymphatic nodules were spherical or oval, and in no instance did the cells of the pale centers tend to infiltrate the surrounding tissues. Sections through the distal third of the appendix showed a large tumor which was lymphoid in type. The mucosa of the appendix had been completely replaced by the neoplasm, and the submucosa and the inner portion of the circular layer of smooth muscle were invaded by the tumor tissue. Tumor tissue was seen also in the mesentery, and in certain instances it encroached on the longitudinal layer of smooth muscle fibers. In the tumor proper the lymphatic nodules showed both numerical and dimensional hyperplasia of a marked degree. The nodules varied in size, but most of them were large, and many occupied an area greater than a single low power field. Some of the smaller nodules were similar to those described in the middle and proximal thirds of the organ. The great majority of them, however, were cytologically identical with the tumor described in case 1 and possessed the same invasive tendencies. In every instance the follicles which showed invasive tendencies were composed of large hypochromatic cells, slightly smaller and more chromatic cells, and hyper-

20. Symmers, D.: Arch. Path. **26**:603, 1938; footnotes 16 and 19.

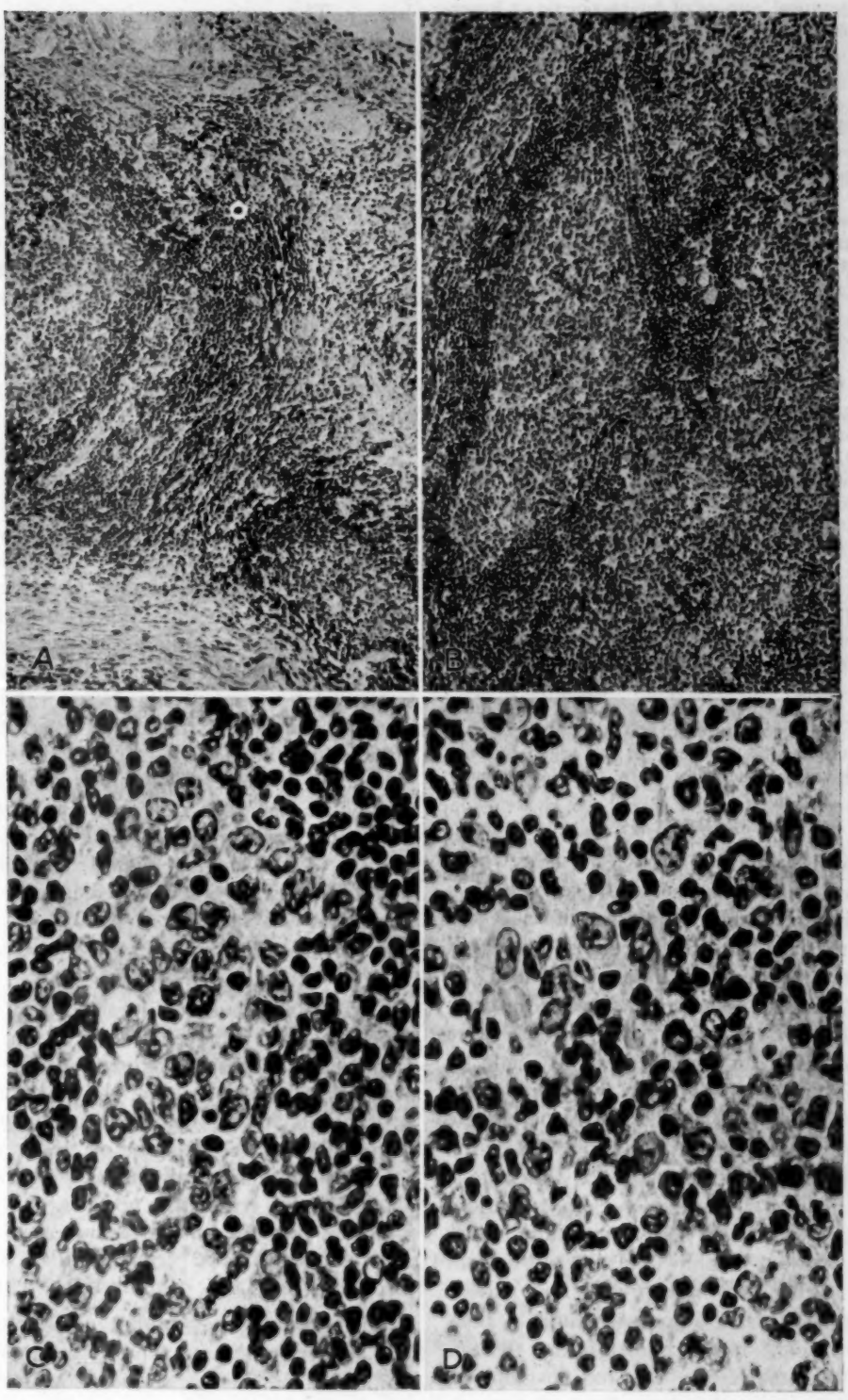

Fig. 2.—*A*, infiltration of the muscular and serous coats of the appendix by lymphatic tissue in case 1; × 110. In the lower portion of the photomicrograph a small bundle of circular muscle fibers is the only remaining evidence of normal appendical structure. *B*, large lymphatic nodules possessing predominant pale centers; × 110. The pale center at the left communicates with that of an adjacent lymphatic nodule. The latter is markedly irregular. *C*, early infiltration of the ring of peripheral lymphocytes by the cells of the pale centers; × 580. There is no sharp line of demarcation. *D*, large hypochromatic cells, smaller and more chromatic cells, and cells resembling medium-sized lymphocytes; × 580. These cells, seen throughout the photomicrograph, constitute the invasive constituents of the tumor.

chromatic cells. The latter cells were similar to those seen in the germinal centers of lymphopoietic or hyperplastic lymphatic nodules.

Following the pathologic study of the appendix, the patient was reexamined for lymphoid enlargements, and none were found. Complete blood studies gave results within normal limits. Examination of the stools, however, did reveal ova of Ascaris lumbricoides, and proper treatment was instituted. Roentgen therapy over the area formerly occupied by the appendix was given. A follow-up examination on Jan. 13, 1945, revealed the patient to be apparently normal in every respect.

CASE 3.—A white man aged 26 consulted Dr. C. V. Tyner, Leaksville, N. C., Jan. 17, 1942, because of abdominal pain. He stated that the pain began on the night of January 13 and gradually became worse, localizing in the right lower quadrant. There had been no similar attack previously. On examination, acute tenderness was present in the right lower quadrant of the abdomen, and there was some resistance in this area. The white blood cell count was 12,400; other laboratory and clinical studies gave results within normal limits. At operation the appendix was found to be adherent to a portion of the omentum and was described as being "acutely inflamed." The organ measured 11 by 0.6 cm. except in the distal third, where the diameter was 0.8 cm. The outer surface was brownish gray and glistening. On section the lumen of the distal third appeared to be completely occluded by grayish white tissue. The microscopic picture was similar to that seen in case 1. The only difference in the gross picture was that in this case the tumor increased the diameter of the organ by only 2 mm. Its invasive tendencies were, however, equally marked.

COMMENT

The clinical findings in these 3 cases would not have led one to suspect the presence of a tumor in the appendix. The pain and the tenderness in the region of the appendix associated with few or no systemic changes would lead the examiner to suspect an inflammatory or obstructive process in the organ. In each of these cases the diagnosis of acute appendicitis was made by the surgeon, and appendectomy was performed. In only 1 instance, however, did pathologic examination reveal infiltration of the wall of the organ by cells other than those of the tumor, and in no case was there evidence of exudation. In the absence of other signs of inflammation, the presence of eosinophils and the fibrosis in case 1 are difficult to interpret. The patient suffered from parasitic infection, but the relation of this to eosinophilic infiltration of the appendix is not established. Marked eosinophilia has been observed in the appendixes of persons describing no symptoms referable to this organ and showing no evidence of parasitic infection.

In case 2 lymphopoietic activity was seen in the pale centers of the proximal and middle thirds of the appendix, while in the distal third —the area of tumor—germinal or lymphocytopoietic centers were present only in small numbers. The great majority of the lymphatic nodules in this area had greatly increased in size and presented numerous bizarre shapes. Their pale centers communicated one with the other and diffusely infiltrated the surrounding tissues. Both cytologically and histologically this tumor fulfilled the criteria for cancer and must be interpreted on morphologic grounds as such.

The first case presented a picture similar to the second, differing from the latter only in the absence of active lymphopoietic or germinal centers in the area of tumor.

The third case was identical with the second except that the tumor showed little evidence of gross enlargement.

It is worthy of special note that in these cases active germinal centers were seen in the proximal end of the organ which were similar to those observed in physiologic lymphoid hyperplasia, while in the area of tumor lymphatic nodules identical with those described in giant follicular lymphoma were present. Also within the tumor were large bizarre follicles showing disruption of their borders, a change in cell type and infiltration of the surrounding tissue. The impression was gained that there was a direct transformation of the follicles from giant follicular lymphoma to cancerous lymphoma identical with that which has been described by Symmers and designated by him as "polymorphous cell sarcoma." Dr. Douglas Symmers studied cases 1 and 2 and concurs in the diagnosis of "polymorphous cell sarcoma."

SUMMARY

Three cases have been described in which the appendix was removed following the development of symptoms simulating those of acute appendicitis. In each instance the appendix showed a localized tumor which was composed of lymphoid tissue. This tumor tissue was characterized by numerical and dimensional hyperplasia of the lymphatic nodules, disruption of follicular outlines and invasion of all coats by large hypochromatic cells, smaller and more chromatic cells and medium-sized lymphocytes.

On morphologic grounds the tumors observed may be interpreted as examples of giant follicular lymphoma of the appendix with direct transformation into "polymorphous cell sarcoma." At the time of operation each appeared to be a solitary tumor, since the appendix was the only structure found to be involved. In the absence of postmortem studies, however, opinion must be withheld regarding the solitary nature of these lesions.

INFLUENCE OF DIETARY DEFICIENCIES AND VARIOUS POISONS ON THE HISTOCHEMICAL DISTRIBUTION OF PHOSPHATASE IN THE LIVER

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Alkaline phosphatase not only plays an essential role in the calcification of bone but also is involved in other metabolic processes.[1] The central position of the liver in the body's metabolism suggested the study of this enzyme as it appears in the liver under various dietary conditions as well as under the influence of various poisons known to lead to severe damage of liver cells. The latter investigation seemed of special interest in view of the well known increase of serum phosphatase in diseases of the liver in man. The histochemical method which permits localization of the enzymatic activity in tissue sections was used. In addition, acid phosphatase was also studied.

The changes in the enzymatic activity of the liver in starvation, in protein depletion and on restriction to choline-free, protein-deficient diets were examined. Damage of the liver was produced by the application of phosphorus, carbon tetrachloride and chloroform.

ANIMALS USED

Young weaning and adult white albino rats, mostly males, of the Wistar strain, and adult white albino mice were used. In addition, the livers of 2 normal rabbits were studied.

DEMONSTRATION OF PHOSPHATASE

Alkaline Phosphatase.—Gomori's[2] method as modified by Kabat and Furth[3] was used. Small alterations of the technic have been described in a previous paper,[4] consisting mainly in a prolongation of the incubation time in the buffer-phosphate mixture. A similar procedure was used by Fell and Danielli.[5] The sections were not counterstained, since in organs containing only small amounts of phosphatase, the counterstain, e. g., basic fuchsin, may interfere with the interpretation of the amount and the localization of enzymatic activity. Krugelis,[6] Bourne[7] and Fell and Danielli have likewise followed this technic.

Acid Phosphatase.—Gomori's[8] method as modified by Wolf, Kabat and Newman[9] was used with small alterations.[4] The slides were routinely incubated for twenty-four hours in the buffer-phosphate mixture, and when no satisfactory staining was obtained, another slide was incubated for forty-eight hours. However, too long incubation leads to diffuse deposition of lead sulfide, obscuring cellular structures. Occasionally even an incubation period of twenty-four hours proved too long. In these cases the incubation time was reduced to five to ten hours. No counterstain was used. While the majority of well stained sections gave clear pictures, some showed marked distortion of the cellular structures without obvious reason. Occasionally no satisfactory staining results were obtained. In all instances, for both alkaline and acid phosphatase preparations, sections of kidney were placed on the same slide. The very constant histochemical picture of alkaline phosphatase activity and the fairly regular picture of acid phosphatase activity in the kidney served as a good control for the quality of the preparation.

RESULTS

Alkaline Phosphatase in the Normal Liver.—The histochemical pattern of alkaline phosphatase in the liver observed in man and different animals has been described by Gomori[10] and Kabat and Furth.[3] Gomori found little activity in the rat. Kabat and Furth observed prominent staining of the epithelium of the sinusoids and of other vessels in the mouse. The bile ducts and the liver cells contained no phosphatase or only traces. In normal young and adult rats I found the nuclei of liver cells stained in a somewhat varying degree (figs. 1 and 5). The nucleoli and the chromatin particles, as well as the nuclear membrane, stained most prominently. The intensity of the nuclear reaction varied

From the Laboratories of the Elizabeth A. Horton Memorial Hospital.

1. (*a*) Kay, H. D.: Physiol. Rev. **12:** 384, 1932. (*b*) Folley, S. J., and Kay, H. D.: The Phosphatases, in Nord, F. F., and Weidenhagen, R.: Ergebnisse der Enzymforschung, Leipzig, Akademische Verlagsgesellschaft m. b. H., 1936, vol. 5, pp. 159-212. (*c*) Albers, H.: Phosphatasen, in Nord, F. F., and Weidenhagen, R.: Handbuch der Enzymologie, ibid., 1940, vol. 1, pp. 408-479.

2. (*a*) Gomori, G.: Proc. Soc. Exper. Biol. & Med. **42:** 23, 1939. (*b*) Takamatsu, H.: Tr. Jap. Path. Soc. **29:** 492, 1939.

3. Kabat, E. A., and Furth, J.: Am. J. Path. **17:** 303, 1941.

4. Wachstein, M.: Arch. Path. **38:** 297, 1944.

5. Fell, H. B., and Danielli, J. F.: Brit. J. Exper. Path. **24:** 196, 1943.

6. Krugelis, E.: J. Cell. & Comp. Physiol. **19:**377, 1942.

7. Bourne, G.: Quart. J. Exper. Physiol. **32:** 1, 1943.

8. Gomori, G.: Arch. Path. **32:** 189, 1941.

9. Wolf, A.; Kabat, E. A., and Newman, W.: Am. J. Path. **19:** 423, 1943.

10. Gomori, G.: J. Cell. & Comp. Physiol. **17:** 71, 1941.

in different animals and also in sections of the same animal. Only rarely was the staining so diffuse that the nuclear structures could not be differentiated. The cytoplasm of the liver cells showed slight staining in most cases. Occasionally irregularly distributed areas of liver cells showed somewhat greater activity. The nuclei of the sinusoids displayed a good amount of activity. The walls of the sinusoids were only occasionally stained. The greatest amount of activity was seen in the periportal fields. The connective tissue surrounding bile ducts as well as that around blood vessels frequently showed marked staining. There was moderate activity in bile duct epithelium while occasionally pronounced staining was seen in the walls of smaller blood vessels. Small bile capillaries were stained only in some instances. Mouse liver presented a similar picture. Here the sinusoidal epithelium was more prominently stained, while the reaction in the periportal fields was less obvious. Occasionally small bile ducts were stained. In the livers of 2 normal rabbits stronger activity was

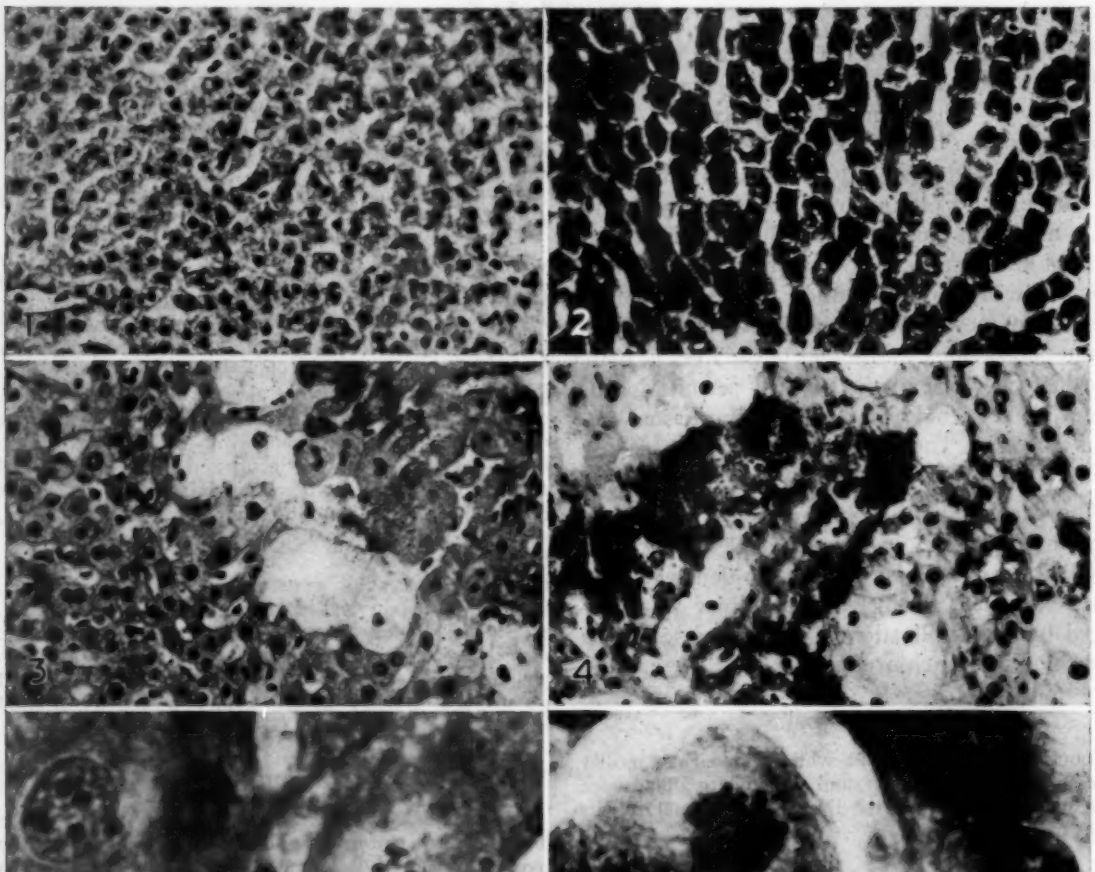

Fig. 1.—Alkaline phosphatase activity in the liver of a normal rat; × 150. The sites of enzymatic activity stain black.

Fig. 2.—Acid phosphatase activity in the liver of a normal rat; × 150. The sites of enzymatic activity stain black.

Fig. 3.—Alkaline phosphatase activity of a mouse liver in chloroform poisoning; × 150. There is marked decrease in activity in large vacuolated cells. The fine granular deposits in the peripheral cells of the central areas of necrosis are calcium deposits.

Fig. 4.—Acid phosphatase activity of a mouse liver in chloroform poisoning; × 150. There is marked decrease in activity in large vacuolated cells. The black deposits in the peripheral cells of the central areas of necrosis are calcium deposits.

Fig. 5.—Alkaline phosphatase activity in the liver of a rat poisoned with carbon tetrachloride; × 600. Nucleoli, chromatin particles and chromosomes show marked activity.

Fig. 6.—Acid phosphatase activity in the liver of a rat poisoned with carbon tetrachloride; × 600. Nucleoli, chromatin particles and chromosomes show marked activity.

noticed. The cytoplasm of the liver cells showed marked staining. In addition, many small bile capillaries, as well as larger bile ducts, showed prominent activity. Alkaline phosphatase activity in the bile capillaries, especially in those in the liver of the rabbit, was first described by Gomori.[10]

Acid Phosphatase in the Normal Liver.—Gomori[8] reported only inconstant staining for acid phosphatase. Wolf, Kabat and Newman[9] with their improved method found regular activity in nuclei and hepatic cells. The nuclei of the liver cells stained deeper. The nuclei of the Kupffer cells showed moderate activity. Acid phosphatase was observed in bile duct epithelium and connective tissue in the portal spaces. My results in normal livers of rats, mice and rabbits were essentially the same (figs. 2 and 6). As previously mentioned, the intensity of the staining reaction cannot always be controlled and depends at least partially on the time of incubation. Sometimes the nuclei showed phosphatase activity in nucleoli and chromatin; at other times they were diffusely blackened. The cytoplasm revealed marked activity, usually in the form of granular brownish to blackish deposits. The activity of the nuclei was not always more pronounced than that of the cytoplasm. Kupffer cells, bile ducts, blood vessels and connective tissue presented less activity than did the hepatic cells. However, definite activity was seen in the bile duct epithelium of the rabbit.

Phosphorus Poisoning.—Nine rats and 4 mice were used. A 1 per mille solution of phosphorus in olive oil was injected intraperitoneally. The dosage for the rats was 0.35 to 0.5 cc. of this mixture per hundred grams of weight, given each time on two to three successive days. The rats survived the last injection for one to three days. The mice (20 to 30 Gm.) received 0.1 to 0.2 cc. of the mixture three times.

The changes seen in rats after phosphorus poisoning have been described by Opie and Alford,[11] by Best, MacLean and Ridout[12] and in detail by Love.[13] Love twenty-four hours after the injection of phosphorus found accumulation of fat at the periphery of the lobules. The fat was uniformly distributed in the form of small droplets. Moderate cell infiltration was seen. Forty-eight hours after the injection he noticed increase in the extent of these changes. The liver contained more fat and showed extensive cytoplasmic vacuolation. Some cells had been completely destroyed, and infiltrating round cells surrounding necrotic cells were seen. These changes reached their height seventy-two hours after the injection. Every cell was now involved, and many mitotic figures were seen.

The changes noticed in my experiments were essentially the same. Most of the liver cells were distended by small fat droplets, and completely necrotic liver cells were frequent in several cases. Mitotic figures were occasionally encountered. Congestion of the vessels was marked in most of the liver, and blood extravasation occurred not infrequently.

Alkaline Phosphatase: Some livers showed about the same amount of activity as normal controls, while in others the cytoplasm of the fatty cells showed little evidence of phosphatase. Completely necrotic cells presented occasionally a slight increase in activity. The nuclei of infiltrating round cells showed mostly marked staining.

Acid Phosphatase: In most cases the cytoplasm of the fatty livers revealed decrease in activity, while normal staining was seen in the nuclei. Since the remaining cytoplasm between the fat droplets gave evidence of acid phosphatase activity, the appearance of these cells was frequently vacuolated. Some of the Kupffer cells stained prominently. Occasionally necrotic cells showed a somewhat stronger reaction than the surrounding fatty cells. Infiltrating round cells showed marked activity.

Chloroform Poisoning.—Twelve rats and 7 mice were used. A chloroform mixture, prepared according to Opie and Alford,[11] containing 1 part chloroform and 2 parts liquid petrolatum, was injected intraperitoneally. The dosage varied between 0.2 and 0.6 cc. of the mixture per hundred grams of weight for each injection. One to two injections were given. The mice received one to three injections of 0.1 cc. of a mixture containing 1 part chloroform and 9 parts liquid petrolatum.

The changes in rats after chloroform poisoning were described by Opie and Alford[11] and quite extensively by Anderson.[14] Six hours after one injection Anderson observed slight microscopic evidence of injury, such as distention of sinusoids and occasionally disruption of the central veins, with increase in fat centrally. Eighteen hours after injection definite necrosis occurred about the central veins. Marked deposition of fat was seen in the necrotic areas and in the periphery. After twenty-four hours there was striking necrosis, cells were swollen and nuclei were pyknotic, small and often absent. Wandering mononuclear cells had invaded necrotic areas to remove dead cells. At the periphery of the lesion, where fatty changes were marked, the cells were extensively vacuolated. After forty-eight hours central necrosis was prominent, and many mitotic figures were noticed. The necrotic areas were invaded by wandering monocytes and also by polymorphonuclear leukocytes. The cellular response, first observed twenty-four hours after the injection, reached its height after the third day. The sensitivity of different animals varied, some showing little and others extensive changes.

The changes observed in my experiments were of a similar nature. The mice showed the more pronounced changes, while the occurrence of necrotic areas in the rats was more irregular and less extensive. In 2 rats four and eight days after the first injection extensive connective tissue fibrosis was observed replacing damaged liver cells. One animal showed, in addition, large areas of calcification. The centrally located necrotic areas were surrounded by large vacuolated cells, frequently with pyknotic nuclei. In several instances the damaged cells mainly showed these changes. Sudan stain demonstrated a varying amount of fat in these cells. Some were completely unstained, while others contained a good amount of stainable fat.

Alkaline Phosphatase: In both rats and mice undamaged cells showed no variation from the norm. Necrotic cells showed either normal activity or apparently some increase in alkaline phosphatase, especially those in the periphery of the necrotic foci. These cells showed dustlike blackish stipples in the cytoplasm (fig. 3). Since, however, control sections for both alkaline and acid phosphatase showed the same black deposits, and von Kossa's stain for calcium was also positive, these black deposits are not indicative of enzymatic activity but represent small calcium deposits. The large vacuolated cells showed distinctly less activity in the cytoplasm, while nuclei, whenever present, showed phosphatase activity. The Kupffer cells in necrotic areas, as well as inflammatory round cells, displayed

11. Opie, E. L., and Alford, L. B.: J. Exper. Med. **21**: 1, 1915.

12. Best, C. H.; MacLean, D. L., and Ridout, J. H.: J. Physiol. **83**:275, 1934.

13. Love, J. G.: Arch. Path. **14**: 637, 1932.

14. Anderson, R. M.: Arch. Path. **14**: 335, 1932.

marked activity in their nuclei. The connective tissue in the fibrotic areas showed mild activity. Mitotic figures revealed that the chromosomes were distinctly active. One animal had marked diffuse increase of phosphatase in undamaged liver cells, especially in the periphery of the lobules.

Acid Phosphatase: The large vacuolated cells presented obvious decrease in activity (fig. 4). The cytoplasm was depleted, while the remaining nuclei presented distinct staining. Most of the necrotic cells revealed a normal or somewhat decreased amount of acid phosphatase activity, while some of the cells, especially those in the periphery of the necrotic foci, showed an apparent increase in this activity. However, as mentioned, these deposits were not due to phosphatase activity, but represented small areas of calcification. The undamaged cells showed normal amounts of phosphatase activity. Chromosomes in dividing cells revealed strong activity. Connective tissue in the 2 animals with replacement fibrosis revealed moderate activity of the nuclei.

Carbon Tetrachloride Poisoning.—Eleven mice and 7 rats were used. A 40 per cent solution of carbon tetrachloride in olive oil was injected intraperitoneally. The mice received varying amounts up to 0.6 cc. of this solution, distributed over several days. Three rats, weighing 200 to 250 Gm., received up to 6 cc. of this mixture. Two rats received 0.3 cc. and 2 others 0.75 cc. of pure carbon tetrachloride subcutaneously.

The effects of carbon tetrachloride on the liver have been described for rats by Lacquet[15] and Cameron and Karunaratne[16] and for mice by Edwards and Dalton.[17] Lacquet as early as six hours after injection of carbon tetrachloride observed degenerative changes in the central fields of the hepatic lobules. The changes were most prominent twenty-four hours after a single injection. There was definite necrosis in central zones, destruction of central veins and infiltration of the necrotic areas by different kinds of cells. The cells were normal in the portal areas, whereas in the midzone there was frequently a ring of clear vacuolated cells, filled with fat, often without any visible cytoplasm or nuclei. Some fat was also noticed in the periphery. Mitotic figures were frequent. The same kind of changes were described by Cameron and Karunaratne, who found the central area of necrosis close to the central vein. The necrotic cells appeared homogeneous, staining deep pink with eosin and presenting pyknotic and degenerative nuclei. They were bordered by enlarged balloon-like cells, which were empty except for a few fine threads between the cell border and the small dark nucleus. This appearance was not due to fat, lipoid or glycogen but represented hydropic degeneration. The cells in the periphery contained a few fat globules. Mitotic figures were abundant. The changes in mice, described by Edwards and Dalton, were quite similar.

The changes seen in my experiments were of the same kind and distribution. In several mice extensive areas of necrosis of the liver were noted. In these necrotic areas the hepatic cells had dense eosinophilic cytoplasm, while the sinusoidal epithelium was apparently not affected. The necrotic areas frequently showed increased cellularity. In several mice large hydropic cells were seen surrounding the necrotic zones. In 2 they represented the main type of degeneration. Sudan stain revealed small fat droplets in some of these hydropic cells, while others were free of fat. Several mice had marked fatty infiltration in the peripheral zones of the hepatic lobules with only a moderate amount of fat in the central areas of necrosis. Beginning cirrhotic changes were seen in 2 animals that had received 0.4 cc. of the carbon tetrachloride and olive oil mixture, given in five injections over a period of thirteen days. Mitotic figures were frequently encountered. The changes seen in rats were less marked than those in mice, although similar in nature. Large balloon-like cells were more prominent. One liver showed abundance of mitotic figures.

Alkaline Phosphatase: Undamaged cells revealed normal amounts of alkaline phosphatase. In some areas there was even increase in activity, particularly in two livers which did not show areas of necrosis. The necrotic cells contained a normal or slightly increased amount of alkaline phosphatase. In these cells, which were devoid of nuclei, granular deposits were seen, staining slightly stronger than the cytoplasm of undamaged cells. In addition, some cells showed fine black granules. These proved to be small preformed deposits of calcium, similar to those described in chloroform poisoning. Nuclei of Kupffer cells, as well as infiltrating round cells, gave usually a strong reaction. The large vacuolated cells showed no activity in the cytoplasm, while pyknotic nuclei gave a pronounced reaction. Proliferating connective tissue showed fairly strong activity. Mitotic figures revealed distinct staining for alkaline phosphatase (fig. 5).

Acid Phosphatase: The enlarged, hydropic cells showed little activity in their cytoplasm, while the nuclei showed normal activity. The completely necrotic cells revealed frequently slightly stronger staining than the surrounding undamaged cells. Some of these cells were covered by black dustlike particles. These blackish deposits were due to deposition of calcium and did not represent phosphatase activity. Infiltrating round cells and proliferating connecting tissue showed fairly definite activity. Undamaged cells showed normal staining, although cells with marked fatty changes presented decreased activity. The chromosomes in mitotic figures were the seat of marked acid phosphatase activity (fig. 6).

Starvation.—Eleven adult mice and 6 adult and 3 weaning rats were submitted to starvation with access to water. Of the mice, 1 died after two days, 1 after three and 1 after four days. The others were killed four, five and seven days, respectively, after the beginning of the fast. They lost between 17 and 36 per cent of their original body weights. Of the adult rats, 1 died after four and 1 after five days; 3 were killed after four and 1 after seven days. They lost between 19 and 34 per cent of their original body weights. The 3 weaning rats were kept in a state of starvation for three and one-half days. They lost between 24 and 27 per cent of their initial body weights.

The livers of all these animals were small and brownish. On microscopic examination, those of the mice were frequently atrophic. The nuclei in many fields appeared larger than those in normal controls. The sinusoids were frequently dilated. The cytoplasm of the liver cells was homogeneous. However, in several of the livers the cells contained small fat droplets. In some of the livers fat could not be demonstrated with specific stains, while in others it was present in small amounts. Similar changes were observed in the rat livers. They contained, however, hardly any stainable fat.

Alkaline Phosphatase: In all the livers of the mice slight to moderate increase in alkaline phosphatase was

15. Lacquet, A. M.: Arch. Path. **14**: 164, 1932.

16. Cameron, G. R., and Karunaratne, W. A. E.: J. Path. & Bact. **42**: 1, 1936.

17. Edwards, J. E., and Dalton, A. J.: J. Nat. Cancer Inst. **3**: 19, 1942.

present (fig. 7). The cytoplasm showed more phosphatase activity than was seen in the livers of control animals. Occasionally bile ducts and capillary walls stained prominently. The length of starvation was apparently of no influence, since one liver showed more phosphatase after two than another after seven days. In the livers of 5 adult rats no increase in alkaline phosphatase was observed. One adult rat and the weaning animals had a slight increase.

Acid Phosphatase: Both rat and mouse livers contained normal amounts of acid phosphatase.

Complete Protein Depletion.—Twenty-six adult mice and 13 adult and 4 weaning rats were restricted to a protein depletion diet. This diet consisted of 80.6 parts

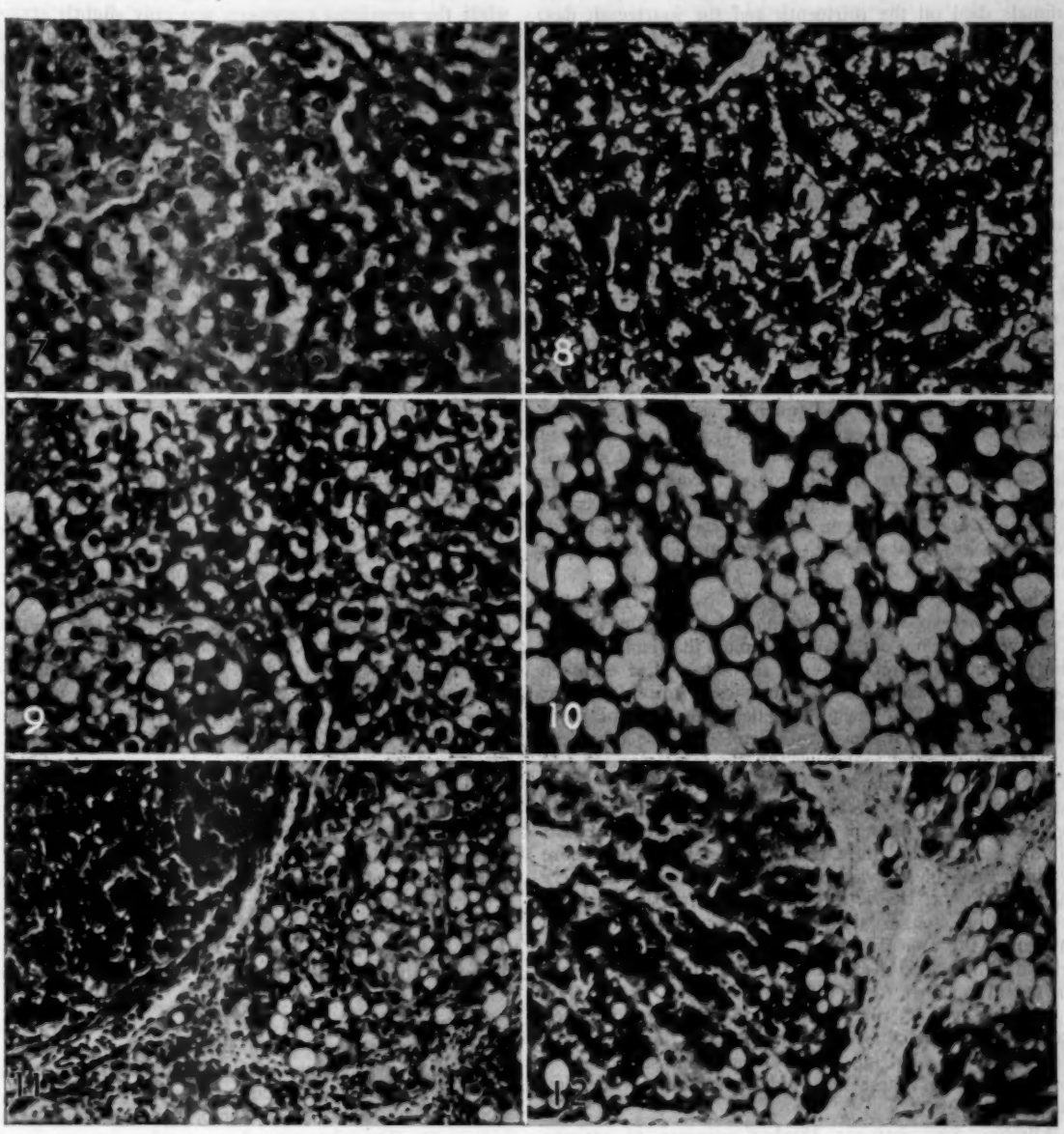

Fig. 7.—Alkaline phosphatase activity of a mouse liver in starvation; × 150. There is moderate increase of activity in the cytoplasm of the atrophic liver cells.

Fig. 8.—Alkaline phosphatase activity in a mouse liver on a protein depletion diet; × 150. There is marked increase of activity.

Fig. 9.—Alkaline phosphatase in the liver of a rat on a protein (methionine)-deficient, choline-free diet; × 150. The fatty cells show decrease, the vacuolated cells increase, in activity.

Fig. 10.—Acid phosphatase in the liver of a rat on a protein (methionine)-deficient, choline-free diet; × 150. Note the marked decrease of activity in the fatty liver cells.

Fig. 11.—Alkaline phosphatase activity in the cirrhotic liver of a rat on a protein (methionine)-deficient, choline-free diet; × 75. There is a striking increase in activity in a fairly fat-free adenomatous area.

Fig. 12.—Acid phosphatase activity in the cirrhotic liver of a rat ten days after restoration of normal diet; × 75. Note the marked contrast between the strong, fairly normal staining of liver cells and the little staining of intervening connective tissue.

sucrose, 15 parts hydrogenated cottonseed oil (Crisco), 4 parts salt mixture no. 2 U.S.P. XII, and 0.4 part bakers' yeast. In addition, the animals received 1 to 2 drops of cod liver oil weekly. This diet was similar to the protein depletion diet 6A used by Sabine and Schmidt.[18] The mice were kept on this diet for periods varying from six to eighteen days. They lost 17 to 35 per cent of their initial body weights. Two of the animals died on the thirteenth and the fourteenth day, respectively; the others were killed by a blow on the neck. The rats received the protein depletion diet for a period varying from eight to forty-six days. Their loss of weight varied between 15 and 52 per cent. Several animals showed a loss of 30 per cent after eight days of the protein depletion diet.

The changes occurring in the livers of animals fed a protein-depleted diet have been described by Elman and co-workers.[19] They noted narrow sinusoids, compressed by wide cords of large liver cells, in dogs and rats. The cytoplasm contained little stainable material, so that it appeared rarefied. There were only a few eosinophilic granules or strands in the otherwise unstained cytoplasm. At times, the eosinophilic strands within the cytoplasm appeared to extend from the nucleus to the cell membrane. There were no distinct round vacuoles. The cell membranes were strongly stained. The nuclei occupied a central position and were never flattened. They were of uniform size and showed the same amount and distribution of chromatin as under normal conditions. Chemical examination demonstrated large amounts of glycogen, a low concentration of nitrogen and a low total content of nitrogen. The fat content was not increased.

The histologic alterations of the livers of some of the mice correspond closely to those described by Elman. They were already observed in animals that had been fed the protein depletion diet only six days; after eighteen days they were marked. In a number of mice, however, which had been fed the protein depletion diet for more than eleven days, the picture resembled more that seen in starvation, with many of the liver cells small and atrophic and the sinusoids widened. In several livers both types of cells were present. Fat stain revealed regularly a fair amount of small fat droplets in most of the cells. The stainable fat was somewhat more distinct than in starvation. Similar observations were made in the rat livers. However, the livers of animals fed the diet longer than twenty-one days frequently presented the appearance associated with hunger. One animal which had been fed the diet for twenty-eight days had marked fatty infiltration of the liver. The 4 weaning young rats kept on the diet for fourteen days showed typical hydropic changes of the liver cells. Fat stains revealed no, or only traces of, stainable fat in the livers of these rats, with the single exception mentioned.

Alkaline Phosphatase: Marked increase of activity was observed in the livers of most of the mice fed the protein depletion diet. The increase was seen in the cytoplasm. The cell border frequently stained as a dark homogeneous brim, while smaller strands and bands of dark material were distributed in the cytoplasm, mirroring the spongy character of these cells. In many cells, however, the cytoplasm appeared diffusely blackened (fig. 8). The strong staining reaction of the cell borders obscured the structure of the sinusoids and made the nuclei of the Kupffer cells hardly recognizable in a number of cases. The intensity of the staining reaction varied. It was only moderate in several instances. A marked increase of activity was also seen in livers in which the cells appeared to be atrophic. The atrophic cells revealed either diffuse staining of their cytoplasm or intense staining of their cell borders while the remaining cytoplasm was only slightly stained. The increase in alkaline phosphatase activity was distinctly more marked in these livers than in the livers of animals which had been submitted to complete starvation. Occasionally the staining reaction became nearly as intense as that seen in the cortical portion of the kidney. The changes observed in rat livers were much less pronounced. In the 4 weaning rats, as well as in several adult rats, no appreciable increase could be noted, although the cell borders stained somewhat more distinctly than those in normal controls. In others, in which liver cell changes of both types (starvation and protein depletion) had occurred, moderate increase of activity was noticed.

Acid Phosphatase: In both mice and rats marked activity of nuclei and cell borders was seen. The cytoplasm of many of the liver cells showed, however, decrease in acid phosphatase activity. It showed frequently only focal staining, similar to that seen in hematoxylin-eosin preparations. Some cells showed normal staining of their cytoplasm. Atrophic cells revealed a normal amount of enzymatic activity.

Restoration Experiments: In two sets of experiments mice were fed the protein depletion diet for eleven and fourteen days, respectively. On these days several mice in each group were killed and their livers examined. These organs showed marked increase in alkaline phosphatase activity. The remaining mice were fed a normal mouse diet (Rockland pellets),[19a] containing 24.8 per cent protein. Five mice were killed after one, two, three, four and six days, respectively. The alkaline phosphatase activity had decreased to normal amounts in three to four days.

Addition of Choline to the Protein Depletion Diet: Four mice were fed the protein depletion diet supplemented by 400 mg. of choline chloride per hundred grams of food for eighteen days. The livers of all 4 animals showed marked increase in alkaline phosphatase activity. There was no significant difference in amount of alkaline phosphatase activity between the livers of these animals and those of controls fed a protein depletion diet without additional choline for the same length of time.

Choline-Free, Protein (Methionine)-Deficient Diet.—Choline-free diets in which the protein, in addition, is deficient in methionine, lead to severe fatty infiltration of the liver.[20] Young weaning rats show in addition in a number of cases severe hemorrhagic necrosis of the kidneys.[21] The changes of renal phosphatase in this condition have been described previously.[4] The livers of 78 animals kept on a choline-free diet for ten to fifteen days were available for study. The source of

18. Sabine, D. B., and Schmidt, H. R.: J. Lab. & Clin. Med. **28**:1117, 1943.

19. Elman, R., and Heifetz, C. J.: J. Exper. Med. **73**:417, 1941. Elman, R.; Smith, M. G., and Sachar, L. A.: Gastroenterology **1**: 24, 1943.

19a. Supplied by the Arcady Farms Milling Co., Chicago.

20. Best, C. H., and Ridout, J. H.: Am. Rev. Biochem. **8**:349, 1939. Best, C. H., and Lucas, C. C., in Harris, R. S., and Thimann, K. V.: Vitamins and Hormones, New York, Academic Press, Inc., 1943, vol. 1. pp. 1.

21. Griffith, W. H., and Wade, N. J.: J. Biol. Chem. **132**: 639, 1940. Griffith, W. H.: J. Nutrition **21**: 291, 1941.

protein in this diet was peanut meal, fibrin or casein. Most of these livers showed fatty infiltration in a varying degree. In some livers nearly all cells contained large fat droplets, which pushed the nucleus to the edge of the cell. In others the fatty changes were only focal, some cells containing one or two small fat droplets. Some livers had only little fatty infiltration, as revealed in special stains. The cells not infiltrated by fat presented a normal appearance or occasionally were atrophic. At times liver cells with scanty cytoplasm and centrally located nuclei were encountered. These cells resembled the hydropic cells seen when animals are given protein depletion diets. In several livers foci of necrotic liver cells were seen. The Kupffer cells in these areas showed degenerative changes. A varying amount of infiltration by round cells and polymorphonuclear leukocytes and proliferation of histiocytes was seen in necrotic foci. Periportal fields frequently showed striking infiltration by round cells. Necrosis of liver cells on a dietary basis was first described by György and Goldblatt.[22]

Alkaline Phosphatase: Nuclei of fat-containing cells, as well as the cells of the vascular endothelium, gave a normal reaction. Wherever large fat droplets had been present, no staining was seen. However, the cytoplasm surrounding fat droplets gave the faint reaction seen in the cytoplasm of normal livers. Cells that were not the seat of fatty infiltration showed either a normal or an increased amount of alkaline phosphatase. The increase was sometimes pronounced. It was observed in atrophic cells as well as in cells resembling the hydropic cells of protein depletion. Specific stains showed this type of cell to contain no or comparatively little fat. In several livers areas of marked fatty infiltration contrasted with areas in which vacuolated cells showed increase of alkaline phosphatase but contained only little fat (fig. 9). Similar to the findings with protein depletion diets, the hydropic cells showed, however, not always increased amounts of alkaline phosphatase. In necrotic areas the infiltrating round cells, as well as proliferating histiocytes, showed great activity and obscured the remnants of necrotic liver cells. In these there was apparently no increase in alkaline phosphatase or even some decrease.

Acid Phosphatase: A striking feature was the depletion in acid phosphatase in all cells containing large fat droplets, whereas the nuclei gave a normal reaction (fig. 10). Those portions of the cytoplasm that were not the seat of fatty changes showed normal activity. Even cells containing large fat droplets which were devoid of activity still had a small brim of black deposits surrounding the empty space. Cells that were not the seat of fatty changes contained a normal amount of acid phosphatase, while hydropic cells revealed decreased amounts. Necrotic areas showed marked acid phosphatase activity. This was due to the prominent staining of the young connective tissue and infiltrating round cells. In addition, some of the Kupffer cells showed increased activity.

The livers of 22 rats fed protein (methionine)-deficient, choline-free diets for a period of from fifty to one hundred and twenty days were also studied. Most of these livers showed extensive fatty changes, and in a few slight areas of fibrosis were seen. Three livers showed small atrophic cells, while in two livers focal areas of hydropic cells were prominent. The behavior of alkaline phosphatase in the fatty livers did not differ from that in the animals kept on the diet only from ten to fifteen days. Increase in alkaline phosphatase was seen in the livers of the animals showing a starvation picture as well as in a liver showing hydropic cells. There was the same decrease in acid phosphatase activity in fatty liver cells as in the previously described groups of animals, while normal, as well as atrophic, liver cells showed fairly normal amounts. A slight decrease was seen in the hydropic cells.

Cirrhosis on a Dietary Basis.—The livers of 15 rats restricted to a protein (methionine)-deficient, choline-free diet for periods varying from ninety to one hundred and fifty days were studied. All livers showed distinct progressive cirrhotic changes. The microscopic appearance of these cirrhotic livers corresponded closely to that described by various investigators.[23] The normal lobular structure was destroyed, and smaller and larger areas of liver tissue were separated by fibrous bands. Single liver cells, as well as islands of them, some necrotic, were seen engulfed in the proliferating connective tissue. Many hepatic cells contained large amounts of fat. However, conglomerations of liver cells, obviously representing regenerating liver tissue, showed no, or only very little, fatty infiltration. The livers contained varying amounts of the peculiar yellowish pigment which has been given the name ceroid by Lillie and co-workers.[23f]

Alkaline Phosphatase: The alkaline phosphatase activity of liver cells showing marked fatty infiltration was not different from that in the fatty livers previously described. Cells without fatty changes showed normal to slightly increased amounts of alkaline phosphatase. Mitotic figures, occasionally seen in these livers, had the typical condensation of alkaline phosphatase in the chromosomes. The adenomatous areas, however, in which the liver cells had a fairly normal appearance, frequently disclosed marked increase in alkaline phosphatase activity, so that they stood out as dark areas in the low power field (fig. 11). The connective tissue cells presented moderate staining. Not infrequently the walls of the sinusoids and small bile capillaries were clearly outlined. Ceroid revealed a somewhat varying behavior in different livers. In some it stained nearly black, while in others it had a brownish-yellow color. Control sections without the substrate presented, however, the same amount of staining. Therefore the staining of ceroid does not signify enzymatic activity.

Acid Phosphatase: The acid phosphatase activity of these livers was similar to that seen in fatty livers. Nonfatty cells contained a normal amount of acid phosphatase, while the connective tissue revealed only moderate activity.

Reparation Experiments: Several animals were kept on the diet for one hundred and twenty days, and then biopsies of liver were made. The animals were thereafter placed on a regular rat diet (Rockland pellets for rats) [19a] containing 24.8 per cent protein and 140 mg. of choline per hundred grams and were killed ten days later. Only animals that showed mild cirrhosis survived the operative procedure. The biopsies revealed severe fatty infiltration and moderate fibrotic changes in the livers. Ten days later, the livers of these animals showed nearly complete regression of the fatty changes. Only an occasional liver cell still contained fat droplets. The fibrotic changes, however, were the same. Alkaline and acid phosphatase preparations demonstrated fairly nor-

22. György, P., and Goldblatt, H.: J. Exper. Med. **70**:185, 1939; **75**:355, 1942. György, P.: Am. J. Clin. Path. **14**: 67, 1944.

23. (*a*) Footnote 22. (*b*) Weber, G.: J. Clin. Investigation **20**: 440, 1941. (*c*) Blumberg, H., and McCollum, E. V.: Science **93**: 598, 1941. (*d*) Blumberg, H., and Grady, H. G.: Arch. Path. **34**:1035, 1942. (*e*) Lillie, R. D.; Daft, F. S., and Sebrell, W. H.: Pub. Health Rep. **56**:1255, 1941. (*f*) Lillie, R. D.; Ashburn, L. L.; Sebrell, W. H.; Daft, F. S., and Lowry, J. V.: ibid. **57**: 502, 1942.

mal enzymatic activity. The contrast between the heavily staining liver cells and the very slightly staining connective tissue in acid phosphatase preparations became extremely prominent (fig. 12). The regression of the fatty changes and the improvement of the cirrhotic condition under similar experimental conditions have been previously described.[24]

COMMENT

Normal liver shows only little alkaline phosphatase activity. The somewhat more distinct reaction in the cytoplasm observed in these experiments was probably due to the longer incubation of the slides in the phosphate-buffer mixture. In agreement with Gomori's findings, normal rabbit liver showed markedly more activity than mouse and rat liver. Chemical examination for phosphatase reveals very small amounts in rat and mouse liver; different results have been obtained on examination of rabbit liver.[25]

The alkaline phosphatase of liver cells is mainly localized in the chromatin substance of the nuclei. Dividing nuclei observed under different experimental conditions showed alkaline phosphatase activity of the chromosomes. Similar observations in tissue cultures from the heart muscles of chick embryos [26] and in dividing cells of the mouse testicle have been reported.[6] In good, not overstained preparations it can be seen that acid phosphatase is likewise localized in the chromatin substance of the nuclei and that it is concentrated in the chromosomes of dividing nuclei. The markedly higher amount of acid phosphatase in normal liver is in full agreement with the results of chemical examinations.[25]

In complete starvation the liver cells were small and the sinusoids dilated. A moderate increase of alkaline phosphatase activity in the cytoplasm of the liver cells was regularly observed in the mouse, while the rat liver showed mostly normal amounts. No changes were observed in the amount of acid phosphatase activity. The livers of fasting mice, but rarely those of rats, showed moderate increase of stainable fat. In the latter animal the fat content of the liver in starvation is only irregularly and slightly increased.[27]

The livers of protein-depleted animals showed changes that had been extensively described by Elman and co-workers [19] in dogs and rats. I observed corresponding hepatic changes in the majority of my animals. If the animals were fed the diet longer, the appearance of the liver changed to that of the starvation liver, probably because the animals would no longer take the food in sufficient quantities. In agreement with Elman, I observed only little stainable fat in the livers of rats in this condition. However, in mice the cells of the liver contained fairly large amounts of stainable fat. Contrary to Elman, Hough and co-workers [28] found in dogs on a protein depletion diet the histologic picture of fatty infiltration and marked increase in liver fat on chemical examination. These contradictory findings may, however, be explained by the difference in the time the animals were kept on the protein starvation diet. Elman and co-workers observed the typical "hydropic" changes in their dogs after three to six weeks, while Hough and co-workers found fatty liver changes in dogs which had been for longer periods on the experimental diet.

Marked increase in alkaline phosphatase was observed especially in mouse liver, while the increase in rat liver was less regular. The increase in alkaline phosphatase activity was most pronounced at the cell borders. Frequently, however, the whole cytoplasm took part in the reaction. Whenever the histologic picture resembled that of starvation, alkaline phosphatase activity was distinctly more elevated than in simple starvation. The acid phosphatase picture had a tendency to imitate the vacuolated appearance of the cells in hematoxylin-eosin stains and suggested a slight to moderate decrease.

Marked fatty infiltration occurred in most of the livers of rats on a choline-free, protein (methionine)-deficient diet. In liver cells containing much fat, reduction of cytoplasmatic phosphatase activity was seen. This was most striking in preparations for acid phosphatase, in view of the large amounts of demonstrable enzyme in the cytoplasm of normal liver. However, since the fat is removed during the process of embedding, it is possible that phosphatase, somehow bound to the fat, is likewise removed. Frozen sections made of unfixed fatty livers and stained for acid phosphatase did not give a clear picture. It was, however, possible to stain these sections with sudan after the acid phosphatase activity had been demonstrated. The fat droplets took the stain in the same way as sections not stained previously for acid phosphatase. The final clarification of this question will have to await chemical determination of the phosphatase activity in fatty livers. Whether the decrease in acid phos-

24. Lowry, J. V.; Daft, F. S.; Sebrell, W. H.; Ashburn, L. L., and Lillie, R. D.: Pub. Health Rep. **56**: 2216, 1941. Lillie and others.[28f]

25. (*a*) Greenstein, J. P.: J. Nat. Cancer Inst. **2**:511, 1942. (*b*) Kay.[18] (*c*) Folley and Kay.[1b]

26. Willmer, E. N.: J. Exper. Biol. **19**:11, 1942.

27. Dible, J. H.: J. Path. & Bact. **35**:451, 1932.

28. Hough, V. H., and Freeman, S.: Am. J. Physiol. **138**:184, 1942. Hough, V. H.; Monahan, E. P.; Li, T. W., and Freeman, S.: ibid. **139**:642, 1943.

phatase is true or only apparent, the changes can be fully reversed by restoring the animals to a normal diet.

An interesting feature of these livers was the presence of cells that were either small and atrophic or marked by hydropic changes of a kind similar to those in cells encountered in livers of protein-depleted animals. The altered cells frequently showed increase in phosphatase activity. In areas of necrosis of liver cells no significant change in phosphatase activity was noticed. Proliferating histiocytes, infiltrating round cells and Kupffer cells, however, revealed strong activity, especially those in acid phosphatase preparations.

In livers with progressive dietary cirrhosis, the histochemical picture was essentially similar with the exception of increased alkaline phosphatase activity in adenomatous areas not containing fat.

Several investigators examined the alkaline phosphatase content of blood serum under different dietary conditions. In starvation the amount of serum alkaline phosphatase decreases in rats,[29] guinea pigs,[29a] dogs [29c,d] and rabbits.[30] In dogs after a prolonged preliminary fast increase in serum phosphatase occurred following carbohydrate ingestion and decrease after consumption of meat.[31] A protein-rich diet lowers the serum alkaline phosphatase activity in dogs and rats.[32] However, Weil and Russell [29b] found that only the ingestion of certain types of unsaturated fatty acids increases the lowered alkaline phosphatase in the serum of fasting rats. Marked increase in serum alkaline phosphatase was found in dogs on protein-depletion as well as in those on protein-deficient diets.[28] The rise in alkaline phosphatase, however, coincided with impairment of liver function as evidenced by the decreased ability of the liver to remove injected rose bengal. The increase of serum phosphatase in these dogs occurred after several weeks only. However, in my experiments, at least in mice and rats distinct increase in liver phosphatase was observed after a few days.

Profound cytoplasmic changes take place in the livers of starved or protein-depleted animals.[33] The increase in alkaline phosphatase indicates probably the intensification of metabolic processes, which are not taking place to that degree under normal conditions. In a few experiments it was seen that additional supplements of choline to the protein depletion diet did not influence the appearance of increased amounts of histochemically demonstrable alkaline phosphatase. However, the changes were fully reversible in the course of only three to four days if the animals were placed on a normal diet.

Marked increase in phosphatase activity was found in the malignant hepatoma of the rat in histochemical preparations,[34] as well as on chemical examination.[35] The mouse hepatoma, however, shows even decreased amounts of this enzyme.[36] This is contrary to the findings in protein depletion. Under these circumstances the mouse liver shows more regular and stronger increase in enzymatic activity than the liver of the rat.

Acute damage of liver cells was caused by the application of phosphorus, chloroform and carbon tetrachloride. The changes seen in rat and mouse liver were essentially the same as those previously described by various authors. Large vacuolated liver cells, commonly described as cells showing hydropic degeneration, were seen in some of the livers in both carbon tetrachloride and chloroform poisoning. A similar type of degenerative change has recently been found to occur frequently in rats fed sulfaguanidine in purified diets.[37] Sometimes these cells contained a fair amount of stainable fat; at other times they were completely devoid of it. Contradictory statements in the literature concerning the fat content of these cells can probably be explained by these not uniform findings. Hydropic degenerated liver cells showed regularly definite decrease of both alkaline and acid phosphatase in their cytoplasm, while the nuclei contained the enzyme in normal amounts. Cells showing a coagulative type of necrosis presented either normal or occasionally moderately increased phosphatase activity. The findings for acid phosphatase were similar, since many of the necrotic cells showed normal or slightly increased amounts of acid phosphatase. Liver cells not injured by these poisons disclosed normal enzymatic activity. The fat-laden liver cells in phosphorus and carbon tetra-

29. (*a*) Bodansky, A., and Jaffe, H. L.: Proc. Soc. Exper. Biol. & Med. **29**:199, 1931. (*b*) Weil, L., and Russell, M. A.: J. Biol. Chem. **136**:9, 1940. (*c*) Freeman, S., and Farmer, C. L.: Proc. Soc. Exper. Biol. & Med. **31**:536, 1934. (*d*) Bodansky, A.: J. Biol. Chem. **104**:717, 1934.

30. Panfrat, J., cited by Morris and Peden.[34b]

31. Bodansky, A.: J. Biol. Chem. **104**:473, 1934.

32. Freeman, S., and Farmer, C. L.: Am. J. Physiol. **113**:209, 1935; footnote 29 *c*.

33. Kosterlitz, H. W.: Biochem J. **38**:xv, 1944.

34. (*a*) White, J.; Dalton, A. M., and Edwards, J. E.: J. Nat. Cancer Inst. **2**:539, 1942. (*b*) Woodard, H. Q.: Cancer Research **3**:159, 1943. (*c*) Kabat and Furth.[8]

35. Greenstein.[25a] Woodard.[34b]

36. Edwards, J. E.; Dalton, A. J., and Andervont, H. B.: J. Nat. Cancer Inst. **2**:555, 1942. Greenstein.[25a]

37. Gross, P.; Axelrod, A. E., and Bosse, M.D.: Am. J. M. Sc. **208**:642, 1944.

chloride poisoning showed decrease of both alkaline and acid phosphatase. The enzymatic activity of the Kupffer cells was mostly unchanged, although there was occasionally some increase in acid phosphatase activity near the necrotic foci. In a few instances there was some increase in alkaline phosphatase in undamaged liver cells. In view of the marked increase of alkaline phosphatase activity in dietary deficiency, this might rather be due to disturbed metabolism of the cells than to the toxic agent itself.

Little is so far known about changes in the phosphatase content of liver cells under abnormal conditions. Takamatsu [2b] stated that necrotic liver cells contain increased amounts of alkaline phosphatase, without giving any further details. This is not confirmed by my experiments which demonstrated no or very slight increase in alkaline phosphatase in liver cells necrotic as a result of chloroform, carbon tetrachloride and phosphorus poisoning and, in occasional cases, in the necrotic liver cells observed in dietary deficiencies.

The proliferating histiocytes showed varying degrees of activity. Sometimes, especially in focal necrotic areas due to dietary deficiency, they presented marked activity. The fibrous strands replacing liver tissue in the cirrhotic livers, on the other hand, showed only little activity. Fell and Danielli [5] found connective tissue very rich in alkaline phosphatase activity in experimental wounds and burns in the acute stages of healing. This activity decreases markedly with the formation of permanent scar tissue. Infiltrating round cells showed large amounts of alkaline and acid phosphatase.

Since Robert's [38] discovery of the increased amount of alkaline phosphatase in the serum in cases of obstructive jaundice, the behavior of this enzyme in the serum in diseases of the liver has been extensively studied. Alkaline phosphatase is more consistently increased in obstructive than in hepatocellular jaundice, with the exception of certain cases in which the jaundice occurs after the use of arsphenamine. It varies in cirrhosis of the liver and is occasionally increased in metastatic carcinoma of the liver and primary liver cell carcinoma without jaundice.[39] These observations have led to a good deal of discussion concerning the origin of the increased serum phosphatase. Complete as well as incomplete obstruction of the bile ducts in the dog leads to prompt and excessive rise in serum phosphatase.[40] This rise is, however, negligible in the cat.[41] The cat is able to excrete phosphatase in the urine, and this may account for the lack of rise in serum phosphatase. In the rat even a decrease of serum phosphatase was observed after ligation of the bile ducts.[42]

Hepatic damage induced by different poisons, such as carbon tetrachloride,[43] phosphorus,[44] chloroform,[44] toluendiamine [45] or neoarsphenamine [43b] or by leptospiral infection [40b] leads to marked rise in serum phosphatase, which is, however, not as great as that in obstructive jaundice. Increase was also found in dogs with Eck fistulas.[43a]

The source of the increased amount of serum phosphatase in damage of the liver is controversial. Previous contentions that in this condition a phosphatase-activating substance in the serum accounts for the increased amount of alkaline phosphatase have not been confirmed.[45] According to Bodansky,[46] serum phosphatase is derived from different organs. The diseased liver may produce more phosphatase. Armstrong and Banting,[47] however, found no decrease in serum phosphatase after extirpation of the liver and removal of all organs which contain large amounts of phosphatase. There was even rise of serum phosphatase in hepatectomized animals. They concluded that the bones must be the origin

38. Roberts, W. M.: Brit. M. J. **1**:734, 1933.

39. (*a*) Meranze, T.; Meranze, D. R., and Rothman, M. M.: Rev. Gastroenterol. **6**:254, 1939. (*b*) Gutman, A. B.; Olson, J. B.; Gutman, E. B., and Flood, C. A.: J. Clin. Investigation **19**:129, 1940. (*c*) Gutman, A. B., and Hanger, F. A.: M. Clin. North America **25**:837, 1941. (*d*) Lichtman, S. S.: Diseases of the Liver, Gallbladder and Bile Ducts, Philadelphia, Lea & Febiger, 1942, pp. 275 and 595.

40. (*a*) Armstrong, A. R.; King, E. J., and Harris, R. I.: Canad. M. A. J. **31**:14, 1934. (*b*) Bodansky, A.: Proc. Soc. Exper. Biol. & Med. **31**:1179, 1934. (*c*) Freeman, S.; Chen, Y. P., and Ivy, A. C.: J. Biol. Chem. **124**:79, 1938. (*d*) Schiffmann, A., and Winkelman, L.: Arch. Int. Med. **63**:919, 1939. (*e*) Gutman, A. B.; Hogy, B. M., and Olson, K. B.: Proc. Soc. Exper. Biol. & Med. **44**:613, 1940. (*f*) Sehra, K. B.; Chopra, I. C., and Mukerji, B.: Indian J. M. Research **29**:647, 1941.

41. Cantarow, A.; Stewart, H. L., and McCool, S. G.: Proc. Soc. Exper. Biol. & Med. **35**:87, 1936. Flood, C. A.; Gutman, E. B., and Gutman, A. B.: Am. J. Physiol. **120**:696, 1937.

42. Weil, L., and Russell, M. A.: J. Biol. Chem. **144**:307, 1942.

43. (*a*) Hartmann, F. W., and Schelling, V.: Arch. Path. **18**:594, 1934. (*b*) Bodansky, A.: Enzymologia **3**:258, 1937. (*c*) Drill, V. A., and Ivy, A. C.: J. Clin. Investigation **23**:209, 1934. (*d*) Freeman, Chen and Ivy.[40c] (*e*) Sehra, Chopra and Mukerji.[40f]

44. (*a*) Armstrong, A. R., and King, E. J.: Canad. M. A. J. **32**:379, 1935. (*b*) Bodansky.[43b]

45. Delory, G. E., and King, E. J.: Biochem. J. **38**:50, 1944.

46. Bodansky, A.: Proc. Soc. Exper. Biol. & Med. **42**:800, 1939; footnotes 31 and 43 *b*.

47. Armstrong, A. R., and Banting, F. G.: Canad. M. A. J. **33**:243, 1935.

of phosphatase and that in damage of the liver the excretion of phosphatase is disturbed. These results were confirmed by Maddock, Schmidt and Thannhauser,[48] who found that hepatectomized dogs showed rise in serum phosphatase and were unable to remove intravenously infused alkaline phosphatase from their blood stream. Gould and Shwachman[49] found marked decrease of serum phosphatase in scorbutic animals, which paralleled a similar decrease of enzymatic activity in the osseous system.

Gutman and co-workers[39b] stated that most of the clinical evidence can be explained by retention of the enzyme in the presence of biliary obstruction. However, by comparing the hepatic tissue changes and serum enzymatic activity in human material, Sharnoff and co-workers[50] found the degree of injury to the hepatic cells to be the determining factor for the increase in serum phosphatase. Similar conclusions were reached by Winkelman and Schiffmann.[51]

The experiments reported here do not favor a hepatic origin of the increased amount of serum phosphatase in hepatic damage. The phosphatase content of the liver cells, at least in rats and mice, is small under normal conditions. It is not materially changed when the cells are most severely damaged and necrotic in chloroform and carbon tetrachloride poisoning. On the other hand, in starvation and protein depletion the amount of phosphatase in the liver is frequently much increased. In all species so far examined, serum phosphatase is low in starvation. In dogs restricted to protein depletion diets a rise of phosphatase, mostly moderate in degree, occurs only following impairment of liver function. In this connection it should be mentioned that phosphatase is excreted not only by the liver but also through the kidneys in some species,[41] in the pancreatic juice[52] and in the intestines.[53] Disturbances in calcium and phosphorus metabolism in hepatic disease may influence the production of bone phosphatase.[54]

48. Maddock, S.; Schmidt, G., and Thannhauser, S. J.: Federation Proc. **1**:181, 1942.

49. Gould, B. S., and Shwachman, H.: Am. J. Physiol. **135**:485, 1942.

50. Sharnoff, J. G.; Lisa, J. R., and Riedel, P. A.: Arch. Path. **33**:460, 1942.

51. Winkelman, L., and Schiffmann, A.: Arch. Int. Med. **64**:348, 1939.

52. Nothmann, M.: Bull. New England M. Center **6**:76, 1944.

53. Kosman, A. J.; Kaulbersz, J. W., and Freeman, S.: Am. J. Physiol. **138**:237, 1943.

SUMMARY

In the livers of normal rats, mice and rabbits, alkaline and acid phosphatase are regularly found in the chromatin substance of the nuclei. Acid phosphatase is present in the cytoplasm of liver cells in much larger amount than alkaline phosphatase. The chromosomes of dividing nuclei show marked alkaline and acid phosphatase activity. Increase in cytoplasmic alkaline phosphatase activity is seen in the atrophic liver cells of starved, and the hydropic liver cells of protein-depleted, mice and to a lesser degree in those of rats. Acid phosphatase activity is fairly normal in starvation and somewhat decreased in protein depletion. The fatty livers of rats fed choline-free, protein (methionine)-deficient diets reveal in cells distended by fat droplets an apparent decrease in alkaline and acid phosphatase activity; cells with atrophic or hydropic changes, however, show frequently increased alkaline phosphatase activity. Adenomatous fat-free areas of cirrhotic livers are often the seat of marked alkaline phosphatase activity.

In livers of rats and mice poisoned by phosphorus and carbon tetrachloride, enzymatic activity is decreased in the cells filled with fat. Necrosis of liver cells was induced by injection of phosphorus, chloroform and carbon tetrachloride in rats and mice; it was occasionally observed in the livers of rats on a choline-free, protein (methionine)-deficient diet. The necrotic cells showed either no or only very slight increase in enzymatic activity. Large hydropic, degenerated cells, frequently encountered in carbon tetrachloride and chloroform poisoning, revealed marked decrease in cytoplasmic alkaline and acid phosphatase activity.

The increase in alkaline phosphatase activity observed in starvation and protein depletion suggests the intensification of metabolic processes dependent on the action of this enzyme. Since little change occurs in necrosis of liver cells, it is assumed that the damaged liver itself does not contribute materially to the increased amount of alkaline serum phosphatase observed in different forms of hepatic disease.

54. (*a*) Austoni, B., and Coggi, G.: Presse méd. **42**: 1594, 1934. (*b*) Morris, N., and Peden, O. D.: Quart. J. Med. **6**:211, 1937.

CARCINOMA DERIVED FROM ADULT SEMINIFEROUS EPITHELIUM

A REVIEW OF THE LITERATURE AND A REPORT OF A CASE

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DETROIT

Stevens and Ewing [1] some time ago called attention to a rare testicular neoplasm which had clinical and pathologic features sharply separating it from other cancers of the testicle. They assigned to it the name "adult multicystic adenocarcinoma." An example of this tumor has recently been encountered, and because of the rarity of the tumor a detailed report of the case will be given, together with brief descriptions of others which have been sufficiently well described to warrant inclusion in this group.

REPORT OF CASE

A white man aged 68 entered Receiving Hospital Dec. 18, 1942, with complaints of a swelling of the scrotum and a mass in the upper part of the right thigh. He was unable to state the exact date of onset but said that the scrotal mass had been present for six or seven years. Pain in the thigh, anorexia and a loss of 40 pounds (18 Kg.) had occurred in the two months previous to entry. The essential findings were a nontransilluminable, firm, painless scrotal mass, 25 cm. in diameter, and an irregular enlargement of the right thigh, chiefly in the proximal half of the medial aspect, but with deep masses palpable almost to the knee. Aside from a positive Kline test and consistent bacilluria, the routine laboratory work showed normal conditions. A roentgenogram of the pelvis revealed extensive neoplastic involvement of the right ischium and lesser involvement of the ilium and the femoral head. Roentgenograms of the chest disclosed small metastatic lesions in the right lung. A diagnosis of metastatic carcinoma was returned after a biopsy of the growth in the thigh. The patient did not respond to general supportive measures and died Jan. 11, 1943, approximately seven years after the tumor was first noticed.

Necropsy.—The right testicle was completely replaced by a lobulated fleshy neoplasm measuring 25 by 15 by 15 cm. (fig. 1). The central portion was yellow and soft; elsewhere the mass was firm. No cysts were present. At some points the tunics were adherent, but the overlying scrotal skin was not ulcerated. No epididymis was recognized. The right inguinal canal was dilated by a mass of varicose veins. The opposite testicle, epididymis and vas deferens were normal. Neither the sacral nor the iliac nodes were noticeably enlarged. The largest metastases were present along the fascial plains of the medial part of the right thigh. These nodules had an appearance similar to the primary neoplasm, many showing yellow central necrosis. In the thorax, the metastases were located on the pleural surfaces as well as in the parenchyma and the hilar nodes of both lungs. Here the tumor tissue was less hemorrhagic and displayed a grayish white and yellow color. The masses varied from a few millimeters to 3 cm. in diameter, the largest being present in the hilar nodes. There was complete agenesis of the right kidney and ureter. No ureteral orifice could be found in the bladder.

Microscopic Examination.—The primary tumor showed two distinct histologic patterns: The peritheliomatous (fig. 2) and the cystic (fig. 3). The former was more frequently seen than the latter and was characterized by endothelium-lined spaces surrounded by broad interconnected bands of cells. The endothelial cells were flat and in most areas were supported by a distinct basement membrane. The spaces contained red blood cells, eosinophilic strands or precipitated albuminous material and occasional tumor cells. The individual cells of the surrounding masses varied moderately in size and were usually round or oval (fig. 4). The nuclei tended to be ovoid, frequently were eccentric and for the most part were deeply stained. Variations of nuclear pattern included giant and piriform shapes and a vesicular arrangement of chromatin. No mitoses were recognized. The cytoplasm was generous, sharply outlined and strikingly eosinophilic. No stromal elements were observed.

The cystic pattern was quite as well defined as the peritheliomatous. The spaces were elongate and angular and appeared to be intercommunicating. The cyst cavities were interrupted at some points by papillary projections of the lining cells and at others by protruding knobs of denuded stroma (fig. 5). The cells lining the cystic spaces varied from low cuboid to columnar. The nuclei were vesicular and centrally placed in a relatively small amount of faintly eosinophilic cytoplasm. They were arranged in single layers except at the point of luminal projection, where they became multilayered. As compared with the tumor cells of the peritheliomatous areas, they were smaller and less eosiniphilic and contained smaller, more uniform, less dense nuclei. No transition from the cells lining the spaces to the cells of the peritheliomatous areas could be satisfactorily demonstrated. The stromal cells had a gently undulating fibrillary structure with scattered small, dark, round to spindle form nuclei. These cystic areas resembled somewhat the rete testis. Other portions of the stroma contained unlined spaces, which varied from round to angular in shape and were extremely variable in size. The metastases in the thigh exhibited the peritheliomatous pattern, while those of the lung tended to show a more alveolar arrangement of cells. The cells in both metastatic sites were arrestingly eosinophilic, as in the primary sites, but the nuclei were more vesicular and giant forms more frequent.

From the Department of Pathology, Wayne University, and the Pathologic Laboratories of Receiving Hospital.

1. Stevens, A., and Ewing, J.: Ann. Surg. **88**:1074, 1928.

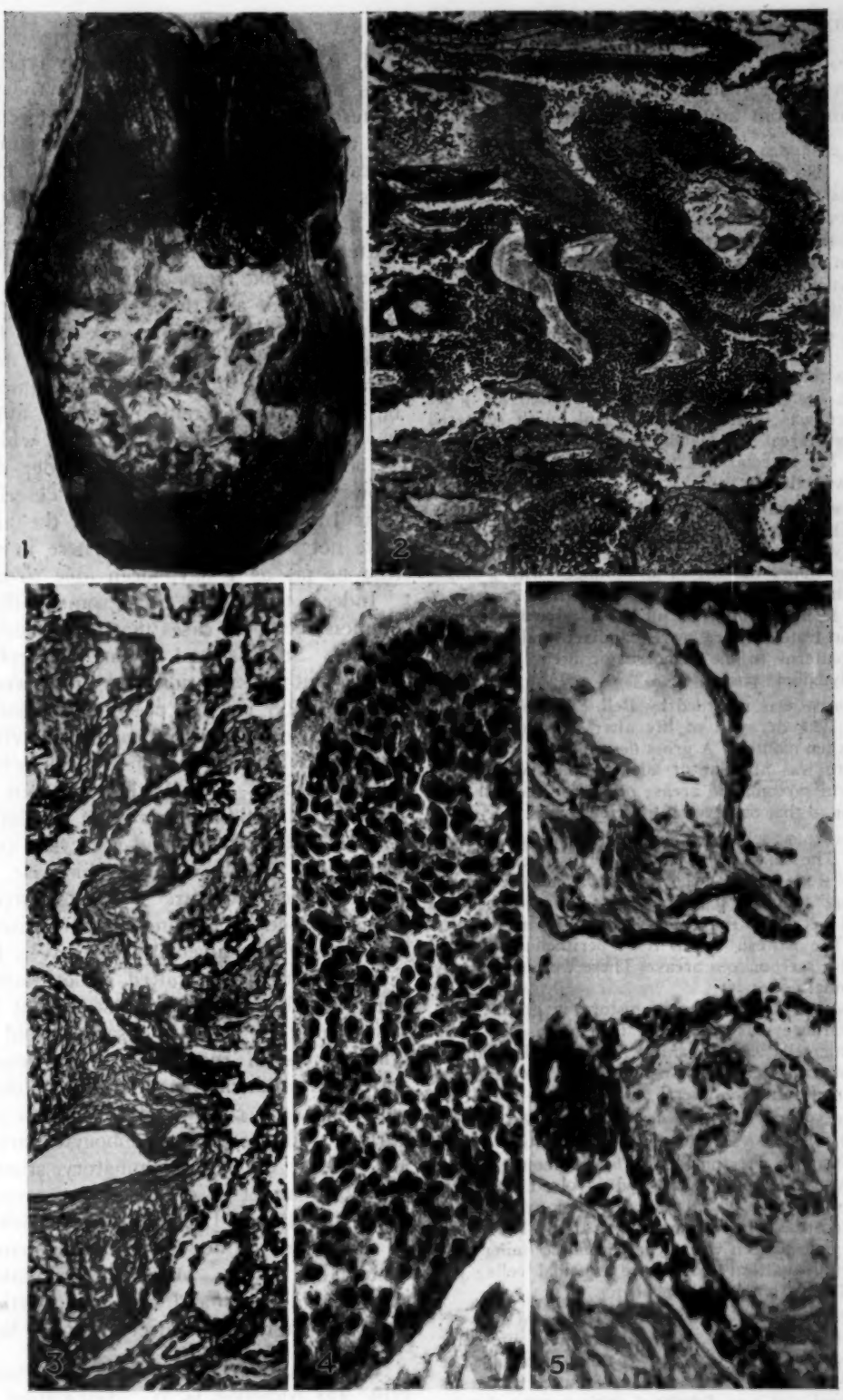

Fig. 1.—Hemisected tumor showing the lobular surface and the central area of necrosis.

Fig. 2.—Peritheliomatous structure showing intercommunicating collars of tumor cells about vascular spaces; × 55.

Fig. 3.—Cystic structure showing lined, angular, slitlike spaces and fibrillary stroma; × 55.

Fig. 4.—The peritheliomatous structure in greater detail, showing tumor cells and the endothelial cells with basement membrane; × 393.

Fig. 5.—The cystic structure in greater detail, showing the character of the lining cells and a multilayered mass; × 393.

SUMMARY OF ADDITIONAL CASES

The following cases appear to be qualified for inclusion in the group of instances of carcinoma originating from adult seminiferous epithelium.

1. The case was reported by Bell.[2] The patient was aged 47. The duration of life after discovery of the tumor was two years; the tumor was a firm, lobulated, fleshy growth, the size of a "closed fist"; the epididymis was free; the testis was nearly destroyed. The neoplasm showed an alveolar pattern of cells resembling spermatocytes, particularly the "cells of the peripheral layer of the seminiferous tubules."

2. This case was recorded by Bell. The patient was aged 52. The duration of life after discovery of the tumor was three years. The right testicle was involved by a firm, fleshy tumor the size of a "closed fist"; the cut surface was mottled red and pink, with scattered hemorrhagic areas; the epididymis was completely destroyed. The alveolar and tubular pattern of cells was identical with that in case 1.

3. Bell reported this case. The patient was 39 years of age. The duration of life after discovery of the tumor was two years. The right testicle was involved by a firm, fleshy tumor the size of an "orange" with a single area of hemorrhage; the epididymis was free. A transition from seminiferous tubules to alveolar pattern was demonstrated; there were papillary and peritheliomatous patterns in the hemorrhagic area; there was scattered lymphoid tissue.

4. This case was recorded by Bell. The patient was aged 47. The duration of life after discovery of the tumor was ten months. A gross description is not given. The pattern was alveolar or adenomatous, with a few spindle form sarcomatoid areas; otherwise the cell type was similar to that observed in previous cases.

5. This case was reported by Bell. The patient was aged 46. The duration of life after discovery of the tumor was three years. The tumor was the size of an "ostrich egg"; it was lobulated, with areas of hemorrhage and necrosis; the epididymis was partially destroyed. The pattern varied from peritheliomatous to tubular, with sarcomatous areas. There were metastases in the inguinal nodes.

Bell reported this case. No history is given. The gross appearance was similar to that in case 5. The characteristic cells were arranged in an alveolar pattern with sarcomatoid areas. The neoplasm had metastasized to lumbar nodes and the liver.

7. This case was recorded by Stevens and Ewing.[1] The patient was 51 years of age. The duration of life after discovery of the tumor was fifteen months. The tumor measured 2.5 by 3 cm. and involved the right testis; it was partially encapsulated and cystic, with opaque solid areas; the epididymis was free. The tumor showed a cystic pattern with hyperchromatic lining cells or an alveolar pattern with large cuboidal cells; the stroma was adult, without lymphocytes.

8. This case was reported by Waterman and Brines.[3] The patient was aged 47. The duration of life after discovery of the tumor was two to three months. The tumor measured 4 by 7 cm. and involved the right testis and epididymis; it was lobulated with a gray, brown and pink surface. The microscopic picture was one of uniform medium-sized cells arranged in tubular and alveolar pattern.

2. Bell, F.: Brit. J. Surg. **13**:282, 1925.

3. Waterman, L., and Brines, O. A.: U. S. Nav. M. Bull. **41**:1690, 1943.

COMMENT

It is hardly possible that this type of tumor was not represented in some of the collections of testicular neoplasms which have been reviewed by numerous observers.[4] For example, the is case 36 of Nicholson's [4b] series. The patien was aged 75. The left testicle was involved by a tumor composed of large cells in alveola pattern, associated with areas containing "numerous slit-like lymphocytic channels." The age of the patient and the histologic description are quite in keeping with the case which I have described, but in the absence of the material or good photographs for study it is impossible to be sure. More recently Hinman and Powell [5] have included in their series cases which appear to be identical with the one under discussion. However, adequate pathologic descriptions of their tumors are not given in the article and have not been reported elsewhere in the literature as far as I have been able to determine.

Indeed there has been some difference of opinion about the cases that are included in this report. Stevens and Ewing [1] accepted Bell's case 4 with reservation and disallowed cases 5 and 6. Later Ewing [6] presumably admitted only the first 3 cases and emphasized the clinical features of relatively late onset, slow growth and late metastasis. He also pointed out the neoplastic destruction rather than displacement of testicular parenchyma and the cystic component which was grossly visible in his case. To him the papillary structure was most striking, although he described, and has an illustration of, the more cellular alveolar areas. Why Bell's last 3 cases were not admitted is not stated. It is true that these cases vary somewhat from the first 3 in that they have sarcomatoid areas of lymphocytes in the stroma. However, Bell [2] stated unmistakably that the sarcomatoid appearance is to be considered only as a histologic variant and that the lymphocytes are present as a result of the inflammatory stimulus and therefore cannot be considered an essential part of the neoplasm. It is evident that Bell believed all 6 tumors to be derived from seminiferous tubular epithelium. While the evaluation of the sarcomatoid areas and the lymphocytic stroma must be left to the future, it should be pointed

4. (*a*) Chevassu, M.: Rev. de chir., Paris **41**:628, 1910. (*b*) Nicholson, G. W.: Guy's Hosp. Rep. **61**: 249, 1907. (*c*) Geist, S. H., and Thalhimer, W.: Ann. Surg. **66**:571, 1917. (*d*) Tanner, C. O.: Surg., Gynec. & Obst. **35**:565, 1922.

5. Hinman, F., and Powell, T. O.: J. A. M. A. **110**:188, 1938.

6. Ewing, J.: Neoplastic Diseases, Philadelphia, W. B. Saunders Company, 1940, p. 863.

ut that the tumor which I have described had either and that the case of Waterman and Brines [3] had an area of ossification which was considered by them to be a degenerative rather than a teratomatous change.

An analysis of the series as it stands shows some interesting similarities and differences. In all the cases in which the side of involvement was mentioned, the right testicle was the site of the primary tumor. The epididymis was involved only after the tumor had reached considerable proportions. This observation strongly supports the contention that these tumors originate in the parenchyma of the testicle. The finding of metastases only in cases of long-standing or fairly large tumors establishes the mildly invasive character of these new growths.

The case here reported exhibits features which have not been previously described. The metastases in the thigh are unique. Likewise, no pulmonary metastases have been previously mentioned. The congenital absence of the right kidney and ureter is of interest, although its exact significance is uncertain. Inasmuch as cancer is known to occur with greater frequency in association with another congenital defect, namely, undescended testis,[7] it might be postulated that this testis was abnormal in some respect before the onset of the cancer. While cystic spaces were present in the microscopic sections, they were not sufficiently large to be visible grossly. This is at sharp variance with Ewing's case in which the spaces imparted grossly a spongelike appearance to a portion of the tumor and the emptying of their fluid content resulted in partial collapse of the tumor.

Unfortunately, already there are several names applied to this group of testicular tumors. Bell [2] favored the term "seminoma," which is in keeping with Chevassu's theory that they were derived from adult seminiferous tubules. Ewing [6] applied a more descriptive term, "adult multicystic adenocarcinoma," but the cysts are not necessarily present, as is seen in this series, and, further, many teratomas have cystic portions. Waterman and Brines [3] employed the term "adenocarcinoma" but were careful to point out that this name had been applied, in the past, to tumors that were derived from embryonal rather than adult cells and more properly might have been called embryonal carcinoma. Hinman and Powell [5] use the term "adult seminoma" and classified the growth in the group of fully differentiated cancers without hormonal activity. While it cannot be said with certainty that the type of tumor under consideration lacks hormonal activity, the experience of Waterman and Brines,[3] as well as the histologic pattern, indicates that no hormone is present. The classification therefore appears to be correct, but the selection of the term "seminoma," about which there has been so much argument, seems less than ideal. Inasmuch, however, as the term adult seminoma is now finding its way into the textbooks of pathology,[8] and since it is desirable to avoid further confusion, it is suggested here that the term be retained and applied in the future to the tumors exemplified in this series.

SUMMARY

Eight testicular neoplasms described in medical literature and one recently studied, and reported here, are thought to be derived from adult seminiferous epithelium. Clinical and histologic features and a review of the terminology and of the position of this group in the newer classifications of testicular tumors suggest that the term "adult seminoma" should, in the future, be confined to it.

7. Gordon-Taylor, G., and Till, A. S.: Brit. J. Urol. **10**:1, 1938.

8. Moore, R. A.: A Textbook of Pathology, Philadelphia, W. B. Saunders Company, 1944, p. 1122.

Case Reports

SIMULTANEOUS OCCURRENCE OF POLYCYSTIC KIDNEYS AND ERYTHROBLASTOSIS FETALIS

Report of Three Cases

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In view of the renewed interest in erythroblastosis fetalis since the discovery of the role played by the Rh factor in the pathogenesis, it is considered worth while to report the occurrence of this condition combined with severe congenital cystic disease of the kidneys in 3 infants born to the same mother. In each of these infants there was also moderate cystic disease of the liver.

REPORT OF CASES

CASE 1.—The patient was a boy born April 16, 1936 at 8:41 a. m.; his heart ceased to beat at approximately 9:30 a. m. the same day (age, 49 minutes). He was slightly premature, the estimated date of delivery being May 22. The body weight was 6 pounds, 9½ ounces (3.0 Kg.) and the length 51 cm. On examination, the liver was palpated 3 fingerbreadths below the costal margin, and the spleen was thought to be palpable. There was a large mass in each flank, which was thought to be a greatly enlarged kidney.

At necropsy the body was cyanotic. The thoracic organs were not remarkable. The liver weighed 270 Gm., and the cut surface was a dark brownish red with numerous irregular fibrous areas along the portal spaces. The spleen weighed 13 Gm. and appeared dark red and firm. Each kidney weighed 120 Gm., and the parenchyma was replaced by numerous cavities, giving the organ the appearance of a sponge. There was diffuse hemorrhagic edema of the scalp, particularly on the right side. The brain was normal, but there was subarachnoid hemorrhage due to a tear in the tentorium.

On microscopic examination the kidneys showed numerous cysts with occasional small areas of normal glomeruli and tubules. The liver showed an increase of fibrous tissue in the portal spaces with numerous cystic bile ducts. Numerous clusters of normoblasts were observed throughout the lobules. Large quantities of hemosiderin were shown in sections stained for iron. Many normoblasts were seen in the blood vessels in various organs. Unfortunately, no section of the spleen was available.

A diagnosis of erythroblastosis fetalis and congenital cystic disease of the kidneys and liver was made.

CASE 2.—The patient was a girl born April 24, 1939, at 11:03 p. m.; death occurred at 12:45 a. m., April 25, 1939 (age, 1 hour and 42 minutes). The expected date of delivery was June 8. The body weight was 5 pounds, 12 ounces (2.6 Kg.) and the length 49 cm.

At necropsy, the liver and the spleen were not weighed, but the weights were estimated as 150 and 10 Gm., respectively. The cut surface of the liver was dark red, and irregular fibrous areas were seen. Each kidney weighed approximately 125 Gm. and showed a spongy, honeycomb structure. The lungs showed partial atelectasis. The head was not opened.

On microscopic examination the liver and the kidneys presented essentially the same appearance as those in the preceding case except that the hemopoietic centers in the liver were more prominent. The spleen showed numerous hemopoietic centers and many nucleated red blood cells in the pulp. The lungs were atelectatic, and many nucleated red cells were seen in the blood vessels. Large deposits of hemosiderin were found in the liver with the iron stain.

A diagnosis of erythroblastosis fetalis and congenital cystic disease of the kidneys and the liver was made.

CASE 3.—The patient was a girl born July 26, 1943 at 2:13 p. m.; death occurred at 4 p. m. the same day (age, 1 hour and 47 minutes). The expected date of delivery was September 17. The weight was 5 pounds, 5 ounces (2.4 Kg.) and the length 46 cm. No note was made of the physical findings except that marked cyanosis was present.

At necropsy the liver weighed 125 Gm., the spleen 20 Gm., the adrenal glands 10 Gm. and the kidneys 110 Gm. and 115 Gm., respectively. The lungs showed marked atelectasis. The urinary bladder was small, the capacity being estimated at about 1 cc. The head was not opened.

On microscopic examination the structure of the liver resembled that in the preceding cases except that hemopoiesis was much more pronounced, the islands of erythroblasts occupying more than a half of the parenchyma. Large quantities of hemosiderin were seen in sections stained for iron. The medullas of the adrenal glands were hemorrhagic. The spleen showed hemopoiesis. The structure of the kidneys was practically identical with that in the previous 2 cases.

A diagnosis of erythroblastosis fetalis and congenital cystic disease of the kidneys and the liver was made.

DATA ON PARENTS OF THE THREE INFANTS

Both parents are healthy young adults; the mother is now (1943) 30 years old and the father two or three years older. They cannot remember any serious illnesses.

The mother's father died of the "flu" in 1918. One grandparent is living, one died of cancer and one died of a "stroke." The father's parents and two grandparents are living; one grandparent died of cancer at an advanced age. All siblings of the parents are living and well. Both parents are sure that there has been no renal disease in either family. One cousin of the father had a baby that died soon after birth, but no other history is available.

Both parents had roentgenograms of the kidneys, ureters and bladder made in 1939. At that time the mother's roentgenogram was reported as showing no abnormality; the report on the father's was as follows: "The right kidney is slightly larger than normal; the left kidney is obscured by gas." No other studies of the urinary tract have been made.

The blood of the parents was examined by Captain Charles P. Larson, Medical Corps, Army of the United States, and his report is quoted here with his permission. The father's red blood cells contain the Rh factor;

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the mother's do not. In September 1943 it was possible to demonstrate the presence of anti-Rh agglutinins in the mother's serum.

The blood picture of the parents (father with the Rh factor in his blood cells, the mother with no Rh factor in her blood cells and with anti-Rh substance in her serum) fits in well with that described as typical in 90 per cent of the cases of erythroblastosis.[1]

COMMENT

It is well known that polycystic renal disease is hereditary, and numerous instances have been reported in which more than one sibling has had the disease.[2] It is a common observation that erythroblastosis often affects several children of the same parents, but I have not found any reports of the two diseases occurring simultaneously. Presumably the association of the two diseases is accidental.

SUMMARY

Necropsy was performed on 3 infants born to the same mother. Each of these infants had erythroblastosis fetalis as well as advanced polycystic disease of the kidneys.

1. Levine, P.; Katzin, E. M., and Burnham, L.: J. A. M. A. **116**:825, 1941.

2. Bell, E. T.: Am. J. Path. **11**:373, 1935.

TUMORS OF THE ISLANDS OF LANGERHANS

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Tumors of the islet tisssue of the pancreas have been known for many years, although sufficient interest in the subject was not aroused until the discovery of insulin by Banting and Best [1] in 1922. The recognition of the effects of an overdose of insulin stimulated considerable interest in these tumors because of the clinical picture which some of them present. Wilder, Allan, Power and Robertson [2] were among the first to point out the clinical correlation between tumors of the island cells and hypoglycemia in their remarkable case of carcinoma of the islands and hypoglycemia. Since then a voluminous literature has appeared.[3]

During the past twelve years we have observed 4 patients with adenoma of the islands of Langerhans, one with a history of hypoglycemia and three with no symptoms referable to the pancreas.

REPORT OF CASES

CASE 1.—On Dec. 12, 1932 a 6 month old white girl was admitted to the Jewish Hospital, New York, because of anorexia since birth, failure to gain weight, constipation and persistent cough. The heart and the lungs were normal. The liver was enlarged, reaching the umbilicus. There were supernumerary digits on both hands.

After ten days' stay in the hospital, the patient showed signs of pneumonia, from which she recovered, and at the end of two weeks she was allowed to go home. The laboratory tests disclosed that the blood sugar during fasting (Jan. 9, 1933) was 29 mg. per hundred cubic centimeters. Two days later it was still 29 mg. The blood sugar rose to 87 mg. an hour after feeding. The levulose tolerance test showed persistent hypoglycemia. The dextrose tolerance test showed low blood sugar during fasting, with a moderate rise following the ingestion of dextrose.

March 4, 1933, the infant was readmitted with the history that she did well for two weeks following her discharge and that she had gained 2 pounds (907 Gm.) in weight. On the day of admission she underwent a convulsive seizure while in the clinic when a sample of blood was withdrawn from the cubital vein. The convulsions lasted several hours and ended in stupor. The blood sugar at this time was 18.3 mg. per hundred cubic centimeters. The spinal fluid was free from cells and showed 11.3 mg. of sugar per hundred cubic centimeters. An intravenous injection of a 50 per cent dextrose solution was given, with some improvement. On the following day, the temperature climbed to 106.2 F., the patient became dyspneic and cyanotic, the pulse was irregular and thready, and death occurred in the presence of profound hypoglycemia.

At autopsy the body was thin, poorly developed and undernourished. There was atrophy of the myocardium, but otherwise the heart was normal. Both lungs were normal. The liver was enlarged and showed fatty infiltration. There was generalized lymphoid and reticuloendothelial hyperplasia.

The pancreas weighed 7 Gm. and grossly appeared normal. No nodules could be seen or felt on the surface. Microscopic examination revealed a small adenoma of islet tissue, separated from the adjoining pancreatic parenchyma by a thin capsule of connective tissue. The cells of the tumor were of uniform size and were arranged in large clusters, which were separated from one another by thin, irregularly formed strands of connective tissue. The capsule showed no cell infiltration. Similarly, the blood vessels were free of tumor cells (fig. 1).

This patient was perhaps the youngest to have an insular tumor associated with hypoglycemia so far reported. Although the tumor was very small and was discovered only on microscopic examination of the pancreas, it was nevertheless sufficiently active to lower the blood sugar to 29 mg. per hundred cubic centimeters a few weeks after admission and to 18.3 mg. just prior to death. There is no doubt that the hypoglycemia was responsible for the death.

CASE 2.—A white man aged 63 years was admitted Aug. 25, 1932, with a chief complaint of frequency of urination and dysuria of nine years' duration. He had been told four years previously that he had diabetes.

Examination revealed nothing unusual except an enlarged prostate. Jan. 3, 1932 cystotomy and bilateral vasectomy were done. Three weeks later the bladder was reopened for the removal of the prostate. It was then discovered that immediately behind this gland there was a large inflammatory mass. The operative wound was therefore closed and the patient returned to bed. November 4, exploratory laparotomy disclosed a tumor mass in the rectosigmoid, and resection of the bowel was done. The patient, however, died three days later. The blood sugar was 103 mg. per hundred cubic centimeters.

At autopsy the pancreas weighed 90 Gm. The free and cut surfaces of the gland appeared perfectly normal.

Microscopically, the parenchyma of the pancreas showed some fatty degeneration and an increase in the interlobular connective tissue. The islands of Langerhans were distinct and somewhat congested. Toward the periphery there was a small circumscribed adenoma which resembled closely the islet tissue of the pancreas in structure and tissue pattern (fig. 2). The tumor cells all stained alike with hematoxylin and eosin and assumed nestlike formations. They had an abundance of cytoplasm, which stained with eosin like the cyto-

From the Departments of Surgery and Pathology, Jewish Hospital.

1. Banting, F. G., and Best, C. H.: J. Lab. & Clin. Med. **7**:251, 1922.

2. Wilder, R. M.; Allan, F. N.; Power, M. H., and Robertson, E. H.: J. A. M. A. **89**:348, 1927.

3. Duff, G. L., and Murray, E. G. D.: Am. J. M. Sc. **203**:437, 1942.

plasm of island cells. The nuclei were round or oval. The stroma was made up of interlacing bands of almost acellular dense connective tissue, which intertwined and divided the parenchyma of the tumor into isolated nests of cells. The pituitary and adrenal glands showed nothing of note.

CASE 3.—A 37 year old white woman was first admitted Jan. 12, 1936, with a chief complaint of postprandial epigastric pain of two years' duration. Two weeks prior to her entry the pain became more acute and was accompanied by vomiting. Gastric analysis revealed marked hyperchlorhydria. A diagnosis of perforating duodenal ulcer was made, and after two weeks of dietary treatment she left the hospital much improved. She was readmitted September 24 because of recurrence of the pain and its failure to respond to treatment. There was considerable tenderness in the right upper quadrant of the abdomen. Roentgen examination revealed a calloused duodenal ulcer. At operation, October 12, a stenosing duodenal ulcer was found, and a gastroenterostomy was made. The postoperative course was uneventful until October 30 when, following a check-up roentgen examination of the stomach, she suddenly vomited, went into shock and died a few hours later.

Laboratory data showed a blood sugar value of 95 mg. per hundred cubic centimeters.

At autopsy the pancreas weighed 100 Gm. and measured 10 by 4 cm. In the body of the pancreas was a circumscribed nodule, 2.3 cm. in diameter. The cut surface of the nodule was pale yellow with occasional gray streaks in its substance. Microscopic examination of the nodule showed slight interacinous and interlobular scarring. The parenchyma of the nodule was composed of cords and sheets of cells embedded in a loose connective tissue and separated from the adjoining pancreatic tissue by a well formed connective tissue of several layers of collagen fibers infiltrated with tumor cells (fig. 3). In some areas the tumor extended beyond the capsule and invaded the pancreatic tissue. There was no evidence, however, of involvement of the blood vessels. The individual tumor cell was large with a dark-staining nucleus and an abundance of light blue-staining cytoplasm. Some of the cells showed mitotic figures. The adrenal and pituitary glands appeared normal.

CASE 4.—An 80 year old white woman was admitted Jan. 20, 1944, because of a fracture of the neck of the right femur and a Colles fracture of the right wrist. The Colles fracture was reduced and the forearm immobilized in a plaster of paris cast. Fixation of the fracture of the femur was accomplished with four pins driven into the femoral neck. Recovery was uneventful until the eleventh postoperative day, when hemiplegia suddenly developed, and the patient died shortly thereafter.

At autopsy the interesting observations were confined to the pancreas, which weighed 80 Gm. Its free surface was smooth and of a yellow-pink color. Arising from the anterior surface of the body was a smooth ovoid mass, 4 by 2.5 by 2 cm. The external surface of the mass was yellow-gray; the consistency was firm (fig. 4). The cut surface presented a homogeneous gray-white glistening appearance. Scattered through the substance of the tissue were small, thin linear red streaks. The entire mass was enveloped in a thin fibrous tissue capsule which merged imperceptibly at one point with the adjoining pancreatic parenchyma.

Microscopic preparations from various portions of the pancreatic nodule exhibited a variegated picture. In some areas the tumor cells showed a striking resemblance to the cells of the islands of Langerhans and were arranged in sheets or in parallel running cords with a delicate supporting stroma (fig. 5). In other places the cells tended toward an acinous or pseudoacinous formation (fig. 6). In still other areas the cells formed small isolated nests, separated from one another by wide bands of partly hyalinized connective tissue in which calcific deposits were apparent. A striking feature of the entire picture was the tumor cell invasion of blood vessels in spite of the fact that the general character of the neoplasm denoted a rather quiescent lesion (fig. 7). The capsule of the tumor was complete throughout and consisted of compact collagen fibers widely infiltrated with tumor tissue. The pancreas itself was normal; the islet cells were numerous and evenly distributed.

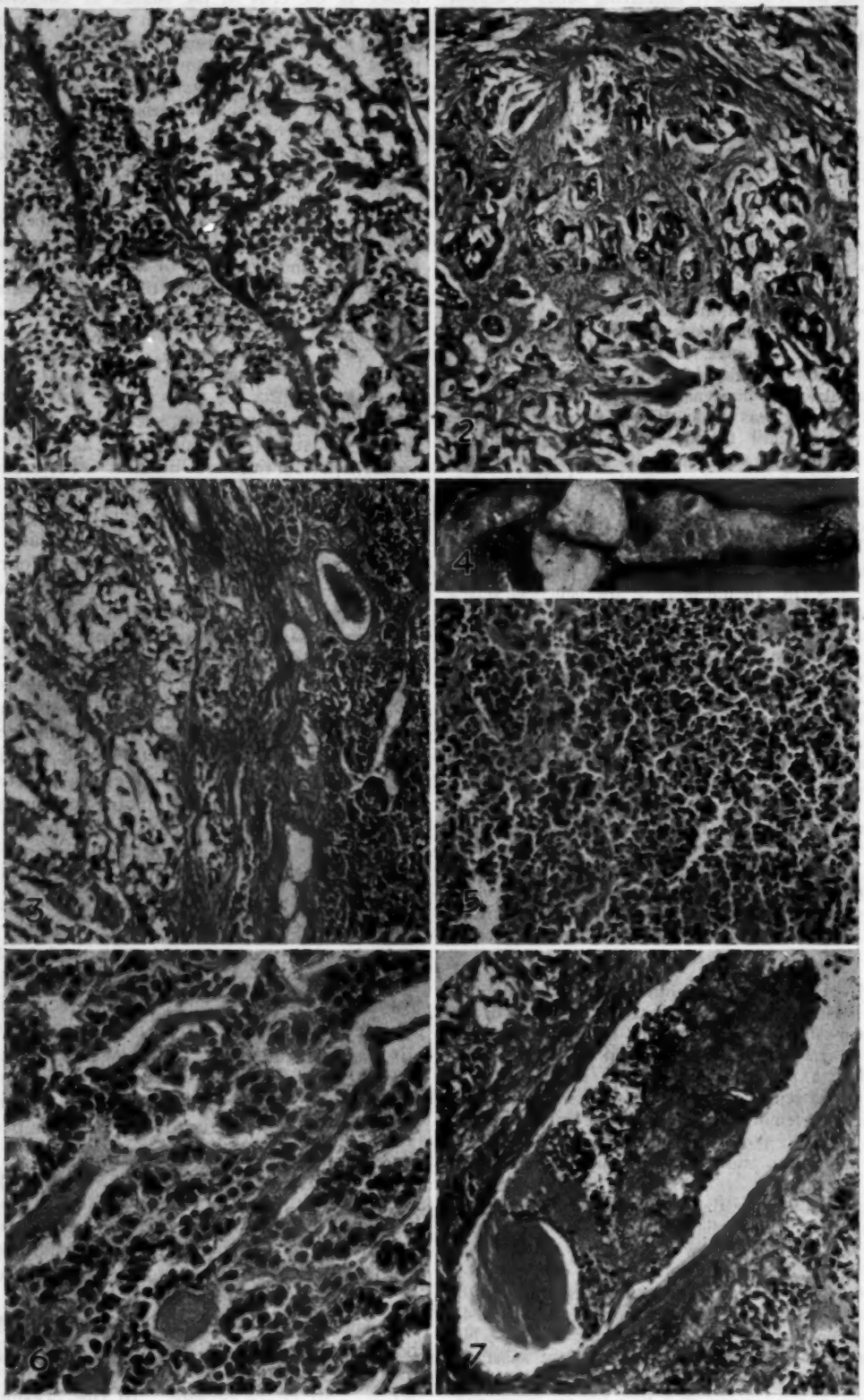
COMMENT

Hypoglycemia in association with tumors of the islands of Langerhans can best be explained on the basis of an increased as well as perverted production of insulin. Physicians are now able to diagnose accurately an attack of insulin shock occurring in a patient who has a tumor of the islands of Langerhans. It is surprising that in many cases the tumor in the pancreas is tiny, and it is rather inconceivable that such a small tumor should account for the hyperinsulinism and the resulting hypoglycemia. This is particularly well illustrated in the first patient in whom the tumor in the pancreas was of microscopic size, yet the hypoglycemia was so severe (18.3 mg. of sugar per hundred cubic centimeters of blood) as to cause rapid death. It has been previously suggested by one of us (J. R.)[4] that in addition to the quantitative excess of insulin produced by the island tumor, there must also be a qualitative change in the character of this secretion so far as it is much more potent than the normal substance regularly secreted by the islands.

It is of interest in this connection that not all islet tumors are associated with hypoglycemia. As a result, many such tumors remain undiscovered during life and are only incidentally disclosed at autopsy. This was true in 3 patients of this series. It has been demonstrated by Bensley and others that not all the cells composing an islet tumor are insulin producing —that only the beta cells are responsible for the elaboration of insulin. It has been suggested, therefore, that adenoma which produces no hypoglycemic symptoms is free from insulin-secreting cells. It has been noted also that the greatest number of tumors of this type, which are symptom free, occur in persons past middle life.

Although adenoma of the islands of Langerhans has been found in patients of all ages, the preponderant number of the patients have been 30 and 50 years of age. In the present series the youngest patient was 6 months old and died

4. Rabinovitch, J., and Barden, F. W.: Am. J. M. Sc. **184**:494, 1932.



(See legends on opposite page)

of severe hypoglycemia. This was one of the youngest patients with adenoma of the islet cells and hypoglycemia recorded. The other 3 patients were 37, 63 and 80 years of age, respectively, and none of them showed clinical evidence of hypoglycemia.

Anatomically these tumors can usually be traced to the island portion of the pancreas. Both in structural arrangement and in cytologic detail they almost regularly have features in common with the islet cells. With few exceptions, the parenchyma of the tumors is made up of specific tumor cells which in appearance approach closely the mother tissue. In the present series the morphologic relations of the various components of the individual growths were very similar to those in the islands of Langerhans, and hence there was no doubt as to the exact source of the neoplasms. Although the structure of the normal islands of Langerhans is almost exactly reproduced in many of the island cell adenomas, nevertheless certain features of the normal pattern become altered. As has been mentioned, the parenchyma of some of the tumors described was made up of sheets or cords of cells supported by a delicate stroma. In others the cells were arranged in clusters or nests separated from one another by thick bands of collagenous fibers, the latter showing hyaline degeneration and calcific deposits. In still other instances the cells assumed an alveolar or pseudoalveolar formation. In case 4 the tumor displayed various combinations of cell forms and tissue patterns. The individual cells composing the tumors were also strikingly similar to those of normal islands. They varied somewhat in size and shape; some were low cuboidal or polyhedral, while others were tall columnar. The cytoplasm was fairly abundant and of a pale blue color. The nucleus was large, round, oval or vesicular in shape and usually centrally placed. Mitotic figures were found in varying numbers in 2 cases.

There has been considerable doubt expressed as to whether adenoma of the islet tissue should be considered as cancerous. Most of these tumors behave in a manner peculiar to noncancerous neoplasms. They rarely extend beyond their site of origin or metastasize to distant organs. They are usually well encapsulated, and the cells show little deviation from the normal. On the other hand, the cells may show mitotic division and invade the capsule and blood vessels; in the latter respect they behave more like cancer. If one is to consider the invasiveness shown in the capsule and the blood vessels, and the mitotic division of the cells as criteria, islet tumors which possess these characteristics should be labeled as cancerous. A similar problem presents itself in adenoma of the thyroid gland, in which the only anatomic evidence of cancer is invasion of the blood vessels. In 2 cases of the present series there were mitotic division and invasion of the capsule and blood vessels. There was no evidence of either local or distal metastasis, nor was there any clinical sign of cancer. All of the tumors were well encapsulated, and the resemblance between the histologic structure of these tumors and that of the normal islands of Langerhans was most striking. From the available literature it appears that true carcinoma of the islet tissue is a rapidly growing tumor which spreads quickly from the pancreas into neighboring structures and metastasizes widely by way of both lymphatic and blood vessels. In the so-called adenoma of the island tissue, aside from the invaded capsule and blood vessels and the occasional presence of mitoses, there is no evidence of metastasis or any striking change in the cytologic structure of the tissue such as has been ascribed to carcinoma.

SUMMARY

Adenoma of the islands of Langerhans was observed in an infant 6 months old, associated with marked hypoglycemia, and in 3 older persons who presented no symptoms referable to the pancreas.

It is still debatable whether tumors of the islands of Langerhans are noncancerous or cancerous. Some present histologic features peculiar to cancer, such as invasion of the capsule and of blood vessels and mitotic division of cells. However, they rarely metastasize to distant organs.

Adenoma of the island tissue not associated with hypoglycemia is rarely discovered during life and is usually seen in older persons.

Explanation of Figures 1 to 7

Fig. 1 (case 1).—Adenoma showing islands of tumor cells with a delicate connective tissue stroma. × 300.

Fig. 2 (case 2).—Islet cell adenoma showing cells arranged in nests with a dense acellular connective tissue stroma. × 150.

Fig. 3 (case 3).—Adenoma consisting of sheets and nests of cells supported by a very delicate connective tissue stroma. There is invasion of the capsule and the adjoining pancreatic parenchyma. × 150.

Fig. 4 (case 4).—Gross appearance of a cut section of an adenoma arising from the body of the pancreas.

Fig. 5 (case 4).—Tumor composed of sheets of cells with a very delicate connective tissue stroma. × 300.

Fig. 6 (case 4).—Area of tumor in which there is a tendency toward alveolar or pseudoalveolar formation. × 200.

Fig. 7 (case 4).—Section showing tumor cell invasion of a blood vessel. × 150.

Laboratory Methods and Technical Notes

A RAPID IRON HEMATOXYLIN STAIN UTILIZING A COMBINED FIXATIVE-MORDANT SOLUTION

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We recently described in a preliminary report a modification of the Heidenhain iron hematoxylin staining technic whereby the process of fixation and mordanting is executed in one step.[1] By this simple combination of the usually separate procedure the entire routine can be completed in the same time as, or even in less time than, the standard Delafield hematoxylin and eosin technic. The brevity of the method is not associated with any decrease in the exactness of the cytologic appearance of the stained material as compared with that obtained by adhering to the classic Heidenhain procedure. We have now extended our original investigations and in the following report present our observations on the effects of staining microscopic sections with hematoxylin after the tissue has been adequately fixed in various solutions containing 1 per cent of ferric alum (ferric ammonium sulfate).

PROCEDURE

Each of the following fixatives was employed as the primary fixing agent:

Bouin's fluid
Solution of formaldehyde U.S.P. 1:10
Lavdowsky's mixture[2]
Zenker's fluid

One gram of ferric alum per hundred cubic centimeters was added to each of the aforementioned solutions. The alum was readily soluble in the concentration used in the acid fixatives but was insoluble in neutral solution of formaldehyde U.S.P. 1:10. However, when the solution of formaldehyde was acidified by the addition of acetic acid sufficient to attain a 0.5 per cent concentration, the ferric alum became readily soluble. The time necessary for the completion of the combined mordanting and fixing was governed by the size of the tissue, and both processes were usually satisfactorily accomplished in one to three days.

After the tissue had been subjected to the combined fixing and mordanting procedure, it was dehydrated by employing either the ethyl alcohol or the dioxane (diethylene oxide) method as advocated for routine preparation of tissue for paraffin infiltration. When alcohol of increasing concentrations was used, the tissue was cleared in cedar oil prior to paraffin infiltration. However, with use of the shorter and less tedious dioxane technic the tissue was placed directly into paraffin after dehydration in three to four changes of dioxane. After the classic methods of embedding and sectioning had been followed, the tissue sections were stained in hematoxylin solution after the slides had been passed from xylene to hematoxylin through intermediate solutions of decreasing concentrations of either dioxane or alcohol. In staining, as in dehydration, there was no important distinction in the cytologic appearance of the stained slide whether the tissue was exposed to one or the other of the two methods. The staining solution employed was the usual Heidenhain hematoxylin preparation made by adding 10 cc. of a ripened 5 per cent solution of hematoxylin in absolute alcohol to 100 cc. of distilled water. Inasmuch as the tissue was mordanted during fixation, the sections were placed directly into the dye after they were hydrated. The tissue absorbed the stain rapidly and overstained in from two to five minutes. Here again the dioxane was more rapid than the alcohol method, although the stained sections obtained by use of both methods were comparable. However, a word of caution relative to the use of either agent (dioxane or alcohol) is to be noted. The tissue should not be retained longer than necessary in dilute solutions of either alcohol or dioxane or in the water wash prior to being placed in the hematoxylin dye. We have found that prolonged water washing may undo the effect of mordanting accomplished by the use of the mordant-fixative combination. The tissue sections should be hastened through the dilute aqueous solutions and the water wash and should not be permitted to remain in the latter for more than one-half to one minute, depending on the thickness of the tissue sections. On the other hand, prolonged washing in aqueous solutions may be resorted to if a hematoxylin stain other than the ferric alum is desired. Thus Delafield's hematoxylin technic or Harris' modification may be employed by merely permitting the tissue which has been previously fixed in a solution containing ferric alum to remain in the water wash for eight to ten minutes before placing the sectioned tissue into a hematoxylin solution provided with its own mordanting agent.

The time necessary to stain the tissue in the hematoxylin solution varies from one to three minutes, again depending on the type of tissue and the thickness of the sections. The tissue is usually overstained during this time, and differential destaining may be accomplished by the use of dilute hydrochloric acid or a saturated aqueous trinitrophenol (picric acid) solution commonly employed

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1. Kupperman, H. S., and Noback, C. R.: Science **98**:591, 1943.

2. Lavdowsky's mixture is made up as follows: solution of formaldehyde U. S. P., 10 parts; 95 per cent ethyl alcohol, 50 parts; glacial acetic acid, 2 parts, and distilled water, 40 parts.

for destaining in the iron hematoxylin technic. Following a satisfactory nuclear stain, any one of the usual counterstains may be utilized for cytologic detail. After the tissue sections have been stained with hematoxylin, there are no special precautions to be emphasized, and the slide may be passed through the ascending series of alcohol or dioxane concentrations according to the customary procedure and manner. After clearing in xylene, clarite or balsam was used as the mounting medium.

RESULTS AND COMMENT

Of the various types of fixatives to which ferric alum had been added, solution of formaldehyde U. S. P. 1:10 (containing 0.5 per cent acetic acid), Lavdowsky's mixture and Bouin's fluid proved to be most adaptable to this modification. The cytologic detail obtained by this modification equaled that observed by following the classic Heidenhain iron hematoxylin method. Zenker's fixative, on the other hand, could not be utilized satisfactorily since the prolonged washing, which is an essential part of the Zenker technic, removed not only the mercury bichloride but the ferric alum from the tissue, thereby nullifying the earlier mordanting action of the fixative.

Studies made in determining the proper interval of time for accomplishing both satisfactory fixation and mordanting showed that when the tissue was fixed it usually contained sufficient mordant for adequate hematoxylin staining. Material that had been placed in the mordant-fixative for two to three years was still appropriately stained by placing the tissue sections in the hematoxylin solution. The time for fixation and mordanting depended on the type and the size of the tissue. Thus small pieces of tissue about 5 mm. square became mordanted in twenty-four to forty-eight hours. Larger pieces about 1 cm. square required seventy-two hours of fixation before the tissue was properly mordanted. All types of tissues were readily adaptable to the combination fixative-mordant procedure. Tissue, however, with a tenacious or relatively impermeable capsule, such as the testis, required a longer period of fixation to accomplish complete mordanting than did tissue without a capsule or one that was glandular in nature, such as the pancreas. Tissues from both mammalian and amphibian sources have been tested with equal success.

Finley successfully adapted this modified hematoxylin stain to invertebrate material and reported that it was excellent for protozoa.[3] He observed that the time necessary to accomplish satisfactory staining was appreciably shorter than that entailed when mordanting was done just prior to staining. In his technic, however, he employed trinitrophenol as the destaining agent, since he found that a slower destaining agent was more adaptable to his routine of staining. Finley employed this modification on ciliates and noted that it was particularly satisfactory for Vorticella. Recently Noble[4] reported in the literature that the combined fixative-mordant proved satisfactory in staining Endamoeba histolytica and other intestinal protozoa. Employing essentially the same technic as we described in our earlier report he found that the time necessary for staining these organisms was shortened appreciably with no apparent sacrifice of cellular detail.

SUMMARY

Through the use of a combined fixative-mordant solution a modified Heidenhain iron hematoxylin stain has been evolved which reduces appreciably the time necessary for performing the standard Heidenhain routine. No mordant is necessary prior to staining, and a satisfactory preparation is obtained in from three to five minutes. The use of the combined fixative-mordant does not exclude utilization of the various other hematoxylin solutions already containing a mordant since the ferric alum mordant can be easily removed in the water wash, thereby enabling the tissue sections to be stained by Delafield's hematoxylin or any other stains not requiring ferric alum mordanting. The addition of the ferric alum to the fixative agent provides a rapid and simple staining procedure adaptable for routine laboratory use. The cytologic detail of the stained preparation is comparable to that observed after the use of the classic and lengthy Heidenhain iron hematoxylin method.

Present address of second author: Long Island College of Medicine, Brooklyn, N. Y.

3. Finley, H. E.: Personal communication to the authors, 1944.

4. Noble, G. A.: Science **100**:37, 1944.

Notes and News

Retirements, Appointments, Etc.—David Marine has retired as chief of the laboratory division of Montefiore Hospital, New York.

C. C. Little has resigned as managing director of the American Cancer Society, and Rear Admiral C. S. Stephenson (M. C.), U. S. N., retired, has been appointed acting director.

The retirement of C. H. Bunting, professor of pathology in the University of Wisconsin since 1908, is announced.

J. H. Lewis has resigned as professor of pathology in the University of Chicago.

E. W. Goodpasture has been appointed dean of Vanderbilt University School of Medicine, Nashville, Tenn., succeeding W. S. Leathers, retired.

James B. McNaught, associate professor of pathology in Stanford University, San Francisco, has been appointed professor of pathology in the University of Colorado, Denver.

Awards.—The Trudeau Medal of the National Tuberculosis Association has been awarded to Florence R. Sabin in recognition of the bearing of her cytologic work on tuberculosis.

Sir Howard W. Florey, Oxford University, England, has been awarded the Lister Medal for 1945 in recognition of his work on the application of penicillin.

Joseph Erlanger, professor emeritus of physiology at the Washington University School of Medicine, St. Louis, has been awarded the certificate of merit and medal for distinguished service of the St. Louis Medical Society in recognition of "his contributions to fundamental knowledge of the cardiovascular and nervous system and to methods of physiological investigation."

Fellowships.—The Finney-Howell Research Foundation, Baltimore, has awarded fellowships for cancer research to Muriel V. Bradley, the radiation laboratory of the University of California; Margaret A. Kelsall, Jackson Memorial Laboratory, Bar Harbor, Maine; Nelicia Maier, Memorial Hospital for the Treatment of Cancer and Allied Diseases, New York; Elizabeth C. Miller, of the department of biochemistry of the University of Wisconsin.

Society News.—At a recent meeting of the Oklahoma Association of Pathologists in Tulsa, Okla., Howard C. Hopps was elected president, Leo Lowbeer vice president, and Béla Halpert secretary-treasurer.

The Virginia Society for Pathology and Laboratory Medicine has been organized with William E. Bray as president, M. B. Beecroft as vice president and T. M. Peery (Alexandria, Va.) as secretary.

Medicolegal Conference and Seminar.—The departments of legal medicine of the medical schools of Tufts College and Harvard and Boston universities, in association with the Massachusetts Medico-Legal Society, will present a six-day program of lectures, conferences and demonstrations having to do with the investigation of deaths in the interests of public safety. Attendance during five of the six days of the course will be limited to fifteen persons who have registered in advance. On one day (October 3) the program will be open to any physician, lawyer, police official or senior medical student who may care to attend. Further information may be obtained from the secretary of the Massachusetts Medico-Legal Society, 25 Shattuck Street, Boston.

Book Reviews

Mass Miniature Radiography of Civilians for the Detection of Pulmonary Tuberculosis (Guide to Administration and Technique with a Mobile Apparatus Using 35 Mm. Film: and Results of a Survey). By Kathleen C. Clark, P. D'Arcy Hart, Peter Kerley and Brian C. Thompson. Medical Research Council, Special Report Series no. 251. Pp. 135, with 51 illustrations. Price 90 cents. London: His Majesty's Stationery Office (New York: British Information Service), 1945.

This brochure of 135 pages describes in meticulous detail the apparatus, the technic, the methods of administration and the results obtained in the mass roentgenographic examination of four selected groups in London, totaling 23,042 persons, in 1943. The classified results afford a basis for more widespread mass roentgenographic examination in the future control of tuberculosis. For first detection roentgenography was accomplished with a 35 mm. apparatus manufactured by Watson. In positive or questionable cases this was followed by conventional-sized films (5 per cent to 10 per cent). A permanent team of eight persons carried out the technical work. The total personnel on the project was thirteen. The general plan was to study 1,500 patients a week. The four groups studied included two factory groups, one office group and a group in a hospital for patients with mental diseases. Persons shown to be tuberculous were referred for medical study. They were first subdivided into treatment and observation groups. Many tables are supplied which give a breakdown of the data on age and employment, home and work contacts, and other points of significance. Roentgen findings are coordinated with clinical signs. The accidental observations of nontuberculous pulmonary and vascular lesions are summarized. A comparison is made with results obtained in eleven other wartime mass surveys in Great Britain previously reported. Many halftone illustrations are used.

CORRECTION

In the article by Dr. William H. Bauer and Dr. Robert A. Fox entitled "Adenomyoepithelioma (Cylindroma) of Palatal Mucous Glands" in the February issue (ARCH. PATH. **39**:96, 1945) the sentence beginning at the bottom of the first column should read:

Mayo and Dockerty, in their first paper listed the "cylindroma" in the second group of their classification of tumors of the salivary glands. They stated that the average grade of malignancy appeared to be higher in this group than in the "mixed tumor" group.